Department of Neurology
Helsinki University Central Hospital
University of Helsinki, Finland

Department of Clinical Physiology and Nuclear Medicine
Helsinki University Central Hospital
University of Helsinki, Finland

THE EFFECTS OF ENTACAPONE ON CARDIORESPIRATORY, AUTONOMIC, AND CLINICAL RESPONSES IN L-DOPA-TREATED PATIENTS WITH PARKINSON'S DISEASE

Jukka Lyytinen

Academic Dissertation

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki in the large auditorium, Haartman Institute, on the 2nd of June, 2006, at 12 noon

Helsinki 2006

Supervised by

Docent Seppo Kaakkola, MD, PhD
Department of Neurology
Helsinki University Central Hospital
Helsinki, Finland

and

Professor Anssi Sovijärvi, MD, PhD
Department of Clinical Physiology and Nuclear Medicine
Helsinki University Central Hospital
Helsinki, Finland

Reviewed by

Docent Tapani Keränen, MD, PhD
Department of Neurology
Tampere University Hospital
Tampere, Finland

and

Docent Olli Raitakari, MD, PhD
Department of Clinical Physiology
Turku University Central Hospital
Turku, Finland

Opposed by

Professor Vilho Myllylä, MD, PhD
Department of Neurology
Oulu University Hospital
Oulu, Finland

ISBN 952-92-0370-5 (nid.)
ISBN 952-10-3135-2 (PDF)

University Printing House
Helsinki, Finland, 2006

To my family

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on three clinical studies, and includes four original publications* referred to in the text by Roman (I–IV) numerals. The data presented in publications III and IV are drawn from the same clinical study. Some unpublished data are also presented.

I Lyytinen J, Kaakkola S, Ahtila S, Tuomainen P, Teräväinen H. Simultaneous MAO-B and COMT inhibition in L-dopa-treated patients with Parkinson's disease. Mov Disord 1997;12:497–505.

II Lyytinen J, Kaakkola S, Gordin A, Kultalahti E-R, Teräväinen H. Entacapone and selegiline with L-dopa in patients with Parkinson's disease: an interaction study. Parkinsonism Relat Disord 2000;6:215–222.

III Lyytinen J, Sovijärvi A, Kaakkola S, Gordin A, Teräväinen H. The effect of catechol-*O*-methyltransferase inhibition with entacapone on cardiovascular autonomic responses in L-dopa-treated patients with Parkinson's disease. Clin Neuropharmacol 2001;24:50–57.

IV Lyytinen J, Kaakkola S, Gordin A, Kultalahti E-R, Teräväinen H, Sovijärvi A. The effect of COMT inhibition with entacapone on cardiorespiratory responses to exercise in patients with Parkinson's disease. Parkinsonism Relat Disord 2002;8:349–355.

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ABBREVIATIONS

AAAD	Aromatic amino acid decarboxylase
ADL	Activities of Daily Living
AE	Adverse event
AIMS	Abnormal Involuntary Movement Scale
ANOVA	Analysis of variance
AUC	Area under the plasma concentration-time curve
BBB	Blood-brain-barrier
BP	Blood pressure
C_{max}	Peak plasma concentration
CI_{95}	95% confidence interval
COMT	Catechol-*O*-methyltransferase
$COMT^{L}$	Low-activity allele of COMT
MB-COMT	Membrane-bound COMT
S-COMT	Soluble COMT
CR	Controlled release
CV	Coefficient of variation
DA	Dopamine
DBP	Diastolic blood pressure
DDC	Dopa decarboxylase
DHPG	3,4-dihydroxyphenylethylene glycol
DOPAC	Dihydroxyphenyl acetic acid
FEV_1	Forced expiratory volume during one second
FVC	Forced vital capacity
HPLC	High pressure liquid chromatography
HR	Heart rate
HRV	Heart rate variation/variability
HVA	Homovanillic acid
H&Y	Hoehn and Yahr scale
LNAA	Large neutral amino acid
MAO	Monoamine oxidase
MAO-A	Monoamine oxidase type A
MAO-B	Monoamine oxidase type B
MHPG	3-methoxy-4-hydroxyphenylethylene glycol
MSA	Multiple system atrophy
NA	Noradrenaline
NMDA	N-methyl-D-aspartate
3-OMD	3-*O*-methyldopa
PD	Parkinson's disease
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion
R-R interval	R-to-R wave interval
SBP	Systolic blood pressure
SN	Substantia nigra
SVES	Supraventricular extrasystole
$t_{1/2}$	Elimination half-life

T_{max}	Time to peak plasma concentration
UPDRS	Unified Parkinson's Disease Rating Scale
Vco_2	Carbon dioxide output
V_E	Minute ventilation
Vo_2	Oxygen uptake
Vo_2/HR	Oxygen pulse
VES	Ventricular extrasystole
W_{max}	Maximum workload

ABSTRACT

Markedly reduced levels of dopamine (DA) in the basal ganglia of the brain, manifesting clinically as rest tremor, muscular rigidity, and slowness/absence of movement, are characteristic of Parkinson's disease (PD). As yet, there is no curative treatment. Among the symptomatic therapies available, oral replacement of DA by its metabolic pre-cursor L-dopa is the most effective. Despite its unsurpassed efficacy, however, L-dopa has significant disadvantages: Its chronic use is associated with motor response complications, e.g., progressive shortening of the duration of drug effect ("wearing-off"), and abnormal involuntary movements (dyskinesia); in addition, extensive peripheral metabolism of L-dopa results in its short half-life, low bioavailability, and reduced efficacy.

In the periphery, oral L-dopa is metabolized mainly by two enzymes: aromatic amino acid decarboxylase (AAAD) and catechol-O-methyltransferase (COMT). Inhibitors of peripheral AAAD, either benserazide or carbidopa, form an integral part of modern L-dopa therapy. They markedly improve L-dopa bioavailability, although its half-life – and therefore its duration of effect in advanced PD – is not significantly altered.

Some nitrocatechol-structured compounds are highly selective, reversible, and orally active inhibitors of COMT. Entacapone, as one example, has all these properties. It improves the pharmacokinetics of L-dopa by increasing its bioavailability and plasma half-life. Entacapone extends the duration of clinical response to each L-dopa dose in patients with advanced PD.

COMT is an important catecholamine-metabolizing enzyme, ubiquitously present in practically all human tissues. It inactivates both endogenous (e.g., noradrenaline, adrenaline, DA) and exogenous catechol-structured compounds, therefore participating in the regulation of the effects of vaso- and neuroactive catechols both at the synaptic level (sympathetic nervous system) and in the circulation. Therapeutic reduction in COMT activity in PD could theoretically lead to adverse cardiovascular reactions, such as hypertension and arrhythmia, especially in circumstances of enhanced sympathetic activity (physical exercise). In addition, patients with PD may be particularly vulnerable to such effects because of the cardiovascular autonomic dysfunction prevalent in these patients, and their use of other drugs that intervene in catecholamine metabolism, such as the monoamine oxidase B (MAO-B) inhibitor, selegiline. Co-administration of entacapone and selegiline may also lead to pharmacodynamic interactions in the central nervous system, either beneficial (improved L-dopa efficacy) or harmful (increased dyskinesia, reduced sleep).

We investigated the effects of entacapone (in 3–5 daily doses of 200 mg each for 1–2 weeks), administered either with or without selegiline (10 mg o.d.), on several safety (blood pressure, heart rate, ambulatory ECG, plasma catecholamine levels, cardiovascular autonomic function, cardiorespiratory exercise responses, adverse events, and dyskinesia) and efficacy (clinical motor disability, L-dopa pharmacokinetics, daily L-dopa dosage) parameters in L-dopa-treated PD patients. A total of 39 patients took part in three double-blind placebo-controlled, crossover studies. Patients had mild to moderate PD, both with (I–IV) and without (III, IV) fluctuations in motor response. In two of the studies (I, II), the cardiovascular, clinical, and biochemical responses were investigated during an L-dopa test, in which the outcome parameters were repeatedly assessed for 6 hours, first after L-dopa only (control), and then after a 2-week treatment with the study drugs (entacapone vs. entacapone + selegiline in I; entacapone vs. selegiline vs. entacapone + selegiline in II). The third study included assessment of standard cardiovascular reflex tests (III) and

spiroergometric exercise testing (IV), first after overnight drug withdrawal (control), and then after a 1-week treatment with entacapone and selegiline as adjuncts to L-dopa. Ambulatory ECG recordings (Holter) were incorporated in two of the studies (II, third study: unpublished results).

The parameters of cardiovascular function (blood pressure, heart rate, ambulatory ECG, cardiovascular autonomics, cardiorespiratory exercise responses) remained unaffected by repeated administration of entacapone, irrespective of selegiline (I–IV). Neither entacapone nor selegiline altered the resting/exercise levels of circulating catecholamines, despite significant changes in their metabolic profile (I, IV).

Entacapone and selegiline, either alone or combined, improved clinical response to L-dopa (I, II). In one of the studies, this improvement was more pronounced during their co-administration than during entacapone alone (I). In the other study, entacapone, selegiline, and their combination caused a similar improvement in L-dopa clinical response; dyskinesias were significantly increased only after their co-administration (II). Entacapone significantly enhanced L-dopa bioavailability. Although selegiline had no effect on L-dopa plasma levels, it caused distinct changes in its metabolic profile (I). Entacapone had no effect on either work capacity or work efficiency in patients treated with L-dopa and selegiline (IV). Entacapone, both with and without selegiline, was generally well tolerated.

Based on the results from these formal studies, the concomitant use of entacapone with selegiline seems to be safe in L-dopa-treated PD patients, also in conditions of maximal physical effort. This is in line with the experience gathered from larger phase III studies, as well as with the general safety profile of the drug. Entacapone had no effect on cardiovascular autonomic regulation. Concomitant administration of entacapone and selegiline may improve clinical efficacy in some patients but may also lead to increased dyskinesia and unwanted adverse reactions, e.g., dizziness, reduced sleep, and nausea. Dopaminergic adverse reactions can be generally controlled by reductions in daily L-dopa dosage.

Keywords: Parkinson's disease, COMT, entacapone, MAO-B, selegiline, hemodynamics, autonomic nervous system, catecholamines, exercise

1. INTRODUCTION

The cardinal clinical features of Parkinson's disease (PD) are poverty/loss of spontaneous and volitional movements (*hypokinesia)*, rest tremor, muscular rigidity, and impairment in maintenance of both balance and posture. PD occurs sporadically, and generally in late life, with an average age of onset of about 60 years. Finland has approximately 750 new cases of PD each year, with the estimated total number of patients around 10 000.

A progressive decline in motor function generally occurs in PD, with many non-motor manifestations (autonomic, cognitive, sensory) possible. Eventually, significant disability ensues in the majority of patients.

The most characteristic brain lesion in PD is the degeneration of pigmented neurons in the dense part of the substantia nigra (SN). These neurons project to the striatum – a key input station of the basal ganglia motor circuits – where they control neuronal firing through tonic release of the dopamine (DA) neurotransmitter. The motor manifestations of PD may be mainly due to the degeneration of nigrostriatal dopaminergic projections and the resulting deficiency in striatal DA.

The causes of PD remain unknown. No definite environmental risk factors have been established, although several putative ones have been suggested. Recent discoveries in genetics have provided much insight into the pathogenesis of PD. Several distinct, although rare, forms of genetically determined parkinsonism have been identified. Defects in certain pathways of protein degradation, oxidative stress, and mitochondrial energy failure have all been implicated as possible mechanisms of nigral cell damage. The etiology of sporadic PD is likely to be heterogeneous, i.e., consisting of a complex interplay of genetic and environmental factors.

In the 1960s, it emerged that L-dopa, a pre-cursor of DA, can cause a dramatic improvement in parkinsonian motor symptoms (1). L-dopa is orally active and can penetrate the blood-brain-barrier (BBB). It is thought that in the brain, exogenous L-dopa is converted to DA, thereby partially restoring the functional dopaminergic deficiency within the striatum.

Although initially highly effective, chronic use of L-dopa was soon observed to correlate with significant clinical problems (2). One is the deterioration of motor effect in a few hours after drug intake. This "wearing off" or "end-of-dose deterioration" has been reported to occur in up to half of the patients after five years of therapy (3). Other more complex and enigmatic motor complications such as "random-off," "freezing," and involuntary movements (dyskinesia) may also appear.

Other symptomatic drug therapies available are DA agonists with direct action on striatal DA receptors, amantadine, and inhibitors of monoamine oxidase B (MAO-B), which reduce the metabolism of DA in the brain. These drugs have some distinct advantages, but generally are clearly less efficacious than L-dopa.

Major shortcomings of L-dopa are rapid peripheral metabolism and a short half-life. Early on, inhibitors of peripheral aromatic amino acid decarboxylase (AAAD) – also called dopa decarboxylase (DDC) – were incorporated into L-dopa therapy. These markedly reduce the peripheral conversion of L-dopa to DA, thus resulting in a major improvement in both efficacy and tolerability. Later, controlled release (CR) formulations of L-dopa/DDC inhibitor were developed to improve the duration of action of the drug, especially in patients with end-of-dose fluctuations. One quite recent strategy for improving the efficacy of L-dopa has been introduction of inhibitors of catechol-*O*-methyltransferase (COMT).

The enzymatic inactivation of catecholamines through *O*-methylation by COMT was first described by Axelrod (4–6). Clinical experience with early COMT inhibitors was disappointing: These compounds showed poor efficacy and were rather toxic (7). In the 1980s, however, a class of highly potent, selective, and orally active inhibitors of COMT was developed (8), including entacapone and tolcapone. These drugs reduce the peripheral metabolism of L-dopa, thereby increasing its half-life and availability for entry into the brain (9). Large, prospective, double-blind clinical trials have shown both entacapone and tolcapone to extend the duration of effect of a single L-dopa dose and to increase the amount of time with motor benefit from L-dopa in PD patients with end-of-dose fluctuations (10–13). Nowadays, entacapone is the only COMT inhibitor widely used in clinical practice, whereas use of tolcapone has been restricted due to its potential for liver toxicity.

A significant amount of COMT activity exists in practically all tissues (14, 15); this enzyme is important in terminating the actions of biologically active – especially circulating – catecholamines of both endo- and exogenous origin. It is conceivable that inhibition of COMT may lead to an increase in concentrations and biological effects (e.g., arrhythmia and hypertension) of its catechol substrates noradrenaline (NA) and adrenaline, especially under conditions of enhanced release (exercise). It therefore seemed prudent to investigate whether the use of entacapone is associated with any significant effects on plasma catecholamine levels, hemodynamics or cardiovascular autonomic function. No such changes have been reported in entacapone-treated healthy volunteers (16, 17). However, the physiology of hemodynamic and cardiovascular autonomic responses of PD patients may differ from that of healthy volunteers in many respects, perhaps due to a high prevalence of autonomic dysfunction (18). In addition, patients are treated with L-dopa, a catecholamine pre-cursor, and many also take selegiline, a MAO-B selective inhibitor, the concomitant use of which with entacapone may lead to pharmacodynamic interactions such as increased dopaminergic adverse effects.

The studies included within this thesis were performed with the aim of further assessing the safety profile of the peripheral COMT inhibitor entacapone in L-dopa-treated patients having mild to moderate PD. The effects of entacapone – both with and without selegiline – on the general tolerability, hemodynamics, cardiac rhythm, and plasma catecholamine profile were investigated. In addition, the effects of peripheral COMT inhibition on cardiovascular autonomic function, on cardiorespiratory exercise responses, and on plasma catecholamine profile at rest and during exercise were investigated in PD patients receiving selegiline in addition to L-dopa.

2. REVIEW OF THE LITERATURE

2.1 The essence of Parkinson's disease (PD)

2.1.1. Epidemiology, neuropathology, and etiology

PD is a chronic degenerative disorder of the nervous system. Reported age-adjusted incidence and prevalence rates of PD range from 9.7 to 13.8, and from 72 to 259 per 100 000, respectively, worldwide (19). With an estimated annual incidence rate of 15 and prevalence of 166 per 100 000 in Finland (20), it is one of the most common causes of neurological disability.

PD is a disorder of late life, and only one-tenth of cases have their onset before the age of 50. Thereafter, an age-related steady increase occurs in both the incidence and prevalence (21); PD occurs slightly more frequently in men than in women (22).

The neuropathologic hallmarks of PD are neuronal loss and depigmentation, and the accumulation of cytoplasmic inclusions called Lewy bodies in SN neurons. Although Lewy bodies are essential to the neuropathologic diagnosis (23), their role in the pathogenesis of PD is unknown. In the human brain, basal ganglia and their connections form a highly complex network of subcortical circuits that play a key role in motor control. The striatum serves as a relay station between cortical motor areas and other parts of the basal ganglia. The SN exerts tonic control on the striatum via the dopaminergic *nigrostriatal tract*. Degeneration and the concomitant loss of function of this tract is thought to be the main pathophysiologic mechanism leading to the motor manifestations of PD. Although PD has been characterized as a "striatal DA deficiency syndrome," it is not entirely clear whether all of the major symptoms result from a deficiency of DA alone, or from a deficiency of DA and other neurotransmitters.

In PD, a widespread neuronal degeneration with Lewy bodies also takes place in the central and peripheral parts of the *autonomic nervous system* (24), including the postganglionic sympathetic and intrinsic cardiac neurons (25, 26). Functional imaging studies have demonstrated that asymptomatic cardiac sympathetic damage is already present in the early stages of the disease (27, 28). The pathophysiology of autonomic dysfunction in PD has not been established. The relative roles of sympathetic vs. parasympathetic insufficiency are also unclear. According to some authors, parasympathetic dysfunction occurs early (29), whereas others suggest a primary involvement of sympathetic autonomics (30).

A diverse array of pathogenetic mechanisms, e.g., defective protein degradation, oxidative cell damage, and failure of mitochondrial complex I, has been suggested to lead to nigral cell damage in PD (31). Aberrant protein degradation by the ubiquitin-dependent proteasomal pathway has been implicated, especially in some of inherited forms of parkinsonism (32).

The etiology of PD remains elusive. Several putative environmental risk factors, e.g., rural living (22), pesticides, and occupational exposure to certain metals (33) have been suggested, but none has been established. The discovery that cytotoxin precursor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes nigral cell death and severe parkinsonism (34) suggests a potential role for environmental toxins in PD. Cigarette smoking is associated with a reduced risk for developing PD (35): A neuroprotective effect of some substance(s) in cigarette smoke has been suggested. Caffeine is another substance the intake of which is inversely related to risk for developing PD (36).

Both epidemiological and genealogic data support the contribution of a significant genetic component in PD (33, 37). In addition, several forms of familial parkinsonism have been recognized in recent years. These are, however, rare, and no specific mutations linked to these entities have been detected in sporadic PD (38).

2.1.2. Clinical manifestations of PD

Parkinsonism can be defined as a clinical syndrome presenting with rest tremor, bradykinesia, rigidity, and postural instability. It actually consists of a heterogeneous group of disorders, of which idiopathic PD is the most common. Since the initial description of symptoms of PD by James Parkinson (39), our view of the clinical characteristics of this disease has been significantly revised, and a plethora of non-motor manifestations have become widely recognized.

Characteristic symptoms

Bradykinesia (slowness of movement) and *akinesia* (poverty, absence, or slowed initiation of movement) may manifest as difficulties with manual dexterity, small handwriting, loss of facial movements with reduced eye blinking, slow and shuffling gait with reduced arm-swing, monotonic speech with loss of volume, and dysphagia. The performance of sequential and repetitive motor acts is especially affected. Parkinsonian *tremor* is typically a 4–6 Hz resting tremor with an initially asymmetric presentation in either one of the upper extremities. Lips, chin, and jaw may be affected as well. Different forms of postural or action tremor or both may also be present. *Rigidity* implies increased resistance to passive movement throughout the range of motion of a joint. Clinically, it can have different qualities, e.g., smooth ("lead pipe") or rachety ("cogwheel"), and may involve both axial and limb muscles. Loss of postural reflexes, stooped posture, rigidity, and bradykinesia/akinesia may all contribute to *postural instability* and the associated gait difficulties which are commonly late manifestations of PD (40). Postural instability is typically refractory to L-dopa therapy. The pathophysiology of the characteristic motor symptoms of PD is highly complex and incompletely understood.

Secondary manifestations

The spectrum of "secondary" (non-motor) manifestations of PD includes dementia, depression, and sleep and autonomic disorders. Most recent data indicate a rather high prevalence (up to 40%) for dementia and depression in PD (41). The neurochemical basis of these symptoms is likely to be complex and to involve multiple neuronal populations. A wide variety of sleep disturbances are also quite frequently encountered (42).

Autonomic manifestations

Autonomic dysfunction in PD has been extensively documented (43, 44). In comparison to healthy controls, autonomic involvement in PD patients is much more frequent (45), reported to occur in as many as 80 to 100% (46, 47). This section will focus on cardiovascular aspects of parkinsonian autonomic failure.

A high prevalence of cardiovascular autonomic dysfunction has been reported in early PD, even in many of the "*de novo*" (unmedicated) patients (48). Despite some conflicting reports (45, 49), most authors have found a positive correlation between cardiovascular

autonomic impairment and either the duration or clinical severity of the disease (50–52). A progression of sympathetic/parasympathetic cardiovascular dysfunction over a 3-year follow-up has also been demonstrated (53).

Dizziness is the most common symptom of autonomic dysfunction in PD (54), found to occur in up to 65 to 80% of patients (46). A 20% prevalence for symptomatic orthostatic hypotension in PD has been reported (55). Parkinsonian patients demonstrate lower values for mean systolic and diastolic blood pressure (BP) than do healthy controls (56), as well as aberrant variation in diurnal hemodynamics (57).

The early reports of autonomic dysfunction in PD based on cardiovascular reflex testing were controversial (54, 58, 59). Later, most studies in parkinsonian patients have shown diminished hemodynamic responses to test stimuli, such as deep breathing, the Valsalva maneuver, orthostatic challenge, and isometric work (18, 47, 49, 60, 61), although some have produced mixed results (45, 62). Aberrant cardiac conduction times, as well as abnormal diurnal patterns of cardiovascular autonomic control have also been demonstrated by use of more sophisticated methods of ambulatory assessment such as computerized analysis of Finger Arterial Pressure, and a spectrum of R to R wave (R-R) interval variation (52, 63, 64). Based on results from studies with de *novo* patients, it seems likely that the early stages of PD are not accompanied by clinically significant dysfunction of cardiovascular autonomic control (29, 65).

A failure in the rise of plasma NA levels has been demonstrated in PD patients with autonomic dysfunction (66). According to some authors, the subgroup of patients with orthostatic hypotension show subnormal basal levels of plasma NA as well as diminished or absent plasma NA responses to orthostatic challenge (46, 67, 68). Others, however, have found no differences in these parameters between PD patients and matched controls (65, 69). Findings on increased hemodynamic sensitivity to exogenous NA in patients with orthostatic hypotension, with an up-regulation of peripheral $\alpha 2$-adrenoceptors are more consistent (67, 68).

2.1.3. Clinical course

The initial symptoms of PD are presumed to be preceded by a pre-symptomatic period of several years. The estimated percentage of nigral cell loss at the onset of clinical signs is 30 to 50%, with a concurrent 80% reduction in striatal DA (70). Thereafter, a progressive, though highly variable, decline occurs in motor function. A less favorable outcome with more rapid functional decline and higher mortality has been suggested for those patients presenting with non-tremor manifestations of PD, for instance, gait disturbance, postural instability, akinetic-rigid syndrome, and cognitive impairment (71–73).

Before L-dopa, the adjusted mortality rate of PD patients was almost three times that of the general population (71). Whether L-dopa has improved life expectancy of parkinsonian patients is still a matter of some controversy (74–77).

The five-stage (I–V) Hoehn and Yahr (H&Y) scale is widely used in grading the clinical severity of PD (71). In an early series of 204 untreated patients, the median duration of disease at stages II through V was 6 to 14 years (71). After introduction of L-dopa therapy, however, the median duration of disease at each of these stages was shown to be markedly longer (9 to 18 years) (78). In addition, a much lower percentage of patients on L-dopa were either severely disabled or dead after a similar time period, when compared to patients without treatment. However, the progressive nature of functional decline in PD, measured by means of the total score of the Unified Parkinson's Disease Rating Scale (UPDRS) (79) in both on- and off-states, remains unaffected by treatment (73).

2.1.4. On the diagnostics of PD

Several clinical features are considered to support the diagnosis of PD (Table 1). However, an early diagnosis is sometimes challenging due to the broad phenotypic variability of PD, the lack of a disease-specific biologic marker, and the overlap of clinical features between different parkinsonian syndromes. No single feature is sufficient in distinguishing PD from other forms of parkinsonism (80). The evidence from *post-mortem* studies has shown that many clinically diagnosed cases do not conform to the neuropathologic criteria for the disease and vice-versa (81–83).

Several sets of clinical criteria for diagnosing PD have been proposed, although most have not been evaluated for their validity and reliability. Some authors have suggested that the diagnostics should rely mainly on excluding patients with atypical features or poor response to L-dopa (84, 85). Regardless of the set of criteria used, a trade-off always exists between sensitivity and specificity (80, 83). The United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD have been the most widely adopted (81) (Table 1).

Table 1. The United Kingdom Parkinson's disease Society Brain Bank clinical Diagnostic criteria, modified from (81).

Inclusion criteria	*Exclusion criteria*	*Supportive criteria*
Bradykinesia	History of	(≥ 3 for definite diagnosis)
and (one or more)	Repeated strokes	Unilateral onset
Rigidity	Repeated head injury	Rest tremor
Rest tremor (4–6 Hz)	Encephalitis	Progressive course
Postural instability	Oculogyric crises	Persistent asymmetry
	Neuroleptic Rx at onset	Excellent response to L-dopa
	More than one relative with PD	Long-lasting response (≥ 5 years) to L-dopa
	Sustained remission	L-dopa-induced chorea
	Strictly unilateral features after 3 years	Long clinical course (≥10 years)
	Supranuclear gaze palsy	
	Cerebellar signs	
	Early severe autonomic involvement	
	Early severe dementia	
	Babinski sign	
	CT: cerebral tumor or hydrocephalus	
	No L-dopa response (large doses)	
	MPTP exposure	

PD, Parkinson's disease; CT, computerized tomography; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

The differential diagnostics constitutes the exclusion of other causes for parkinsonism using medical history, clinical examination, assessment of response to drug therapy, and long-term follow-up (Table 1). After 5 years from the onset of symptoms, the clinical diagnosis is possible in most cases (86). The use of radio- and functional imaging methods, biochemistry, clinical physiology, and neurophysiology may be considered in a selective group of cases, although these are generally unnecessary. [^{18}F]6-fluoro-L-dopa imaging with positron emission tomography directly measures DA terminal integrity in the striatum, but it is seldom used in clinical practice due to its relatively high cost and limited availability. Single photon emission computerized tomography with, for instance, a [^{123}I]β-CIT (2β-carboxymethoxy-3β(4-iodophenyl)tropane) tracer is clinically more feasible, and shows adequate sensitivity in discriminating PD from other causes of parkinsonism (87). Thus far, genetic testing for specific mutations known to cause familial parkinsonism is not routinely warranted in sporadic disease.

Major clinical entities that should be differentiated from PD include essential tremor, vascular & drug-induced parkinsonism, Alzheimer's disease, and Parkinson-plus syndromes, which include multiple system atrophy (MSA), progressive supranuclear gaze palsy, diffuse Lewy body dementia, and corticobasal ganglionic degeneration. In contrast to PD, Parkinson-plus syndromes demonstrate a more widespread neuropathologic involvement and "atypical" clinical features, e.g., early dementia, other cortical signs, severe autonomic failure, marked postural instability, and poor response to L-dopa.

2.2. Treatment of PD

The modern treatment of PD is mainly targeted at enhancing striatal dopaminergic activity. Replacement of striatal DA with L-dopa is the principal type of dopaminergic therapy, whereas the DA receptor agonists are able to "mimic" the actions of DA in the striatum. MAO-B inhibitors reduce the degradation of striatal DA, thereby enhancing its actions. COMT inhibitors improve L-dopa entry into the brain by inhibiting its peripheral catabolism. Some novel experimental drugs have non-dopaminergic modes of action, e.g., the antagonists of N-methyl-D-aspartate (NMDA) or adenosine A_{2A} receptors. A concise review is available of the pharmacological properties of current antiparkinsonian drugs (88).

Functional stereotactic (deep brain) surgery for PD is targeted at specific sites in the basal ganglia or thalami. A modern approach is to insert a deep brain-stimulating device into the target site by a stereotactic method. Surgical treatment has been recognized as having beneficial effects in the symptomatic control of advanced PD (89), but it is generally reserved for cases with disease refractory to drug therapy or for those with functionally disabling motor complications.

2.2.1. L-dopa

The emergence and principles of therapy

In the early 1960s appeared the first report on the anti-akinetic effect of intravenous L-dopa in PD patients (1). It was not until the findings by Cotzias et al., demonstrating the clinical efficacy of high oral doses of both racemic dopa and L-dopa (90, 91), that L-dopa established its position as a major drug in the management of PD.

Concise reviews are available on the pharmacokinetics of L-dopa (92, 93). Orally administered L-dopa is predominantly absorbed from the proximal small intestine by an active, saturable carrier system of large neutral amino acids (LNAAs) (94). It then undergoes

extensive metabolism (92): At least 80% of the dose enters the catecholamine degradation pathways (95). The gut wall is the first barrier, at which the majority of the drug is rapidly metabolized to DA by DDC (96). The oral bioavailability of L-dopa is therefore low (97). After absorption, plasma L-dopa is rapidly catabolized (mostly to DA) with an elimination half-life ($t_{1/2}$) of 0.8 to 1.7 hours (98, 99). In analogy with the gut wall, circulating L-dopa is taken up and transported through the BBB by the LNAA carrier system (100). At both these sites, other LNAAs can competitively inhibit the transport of L-dopa.

In 1967, inhibitors of extracerebral DDC with poor penetration through the BBB were demonstrated to enhance the L-dopa-induced increase in brain DA levels (101). Two effective inhibitors of peripheral DDC became clinically available, namely benserazide [(±)-D,L-seryl-(2,3,4-trihydroxybenzyl)hydrazine] and carbidopa (1-α-methyldopa-hydrazine) (91, 102, 103); their pharmacological and clinical effects are nearly identical (104). The main site of action of DDC inhibitors seems to be the intestinal wall (105). Use of a DDC inhibitor as an adjunct to L-dopa results in reduced formation of peripheral DA with less DA-related peripheral adverse effects, e.g., nausea and vomiting (106). More L-dopa thus becomes available for transport through the BBB into the brain, where decarboxylation to DA can freely occur (93). The bioavailability of L-dopa is much improved, and the clinical benefit is achieved despite drastic reductions in total daily dosage (107).

In the brain, DDC rapidly decarboxylates L-dopa to DA. According to the DA storage hypothesis, this neurotransmitter is then stored presynaptically until its firing-coupled vesicular release into the synaptic cleft (108). Evidence is unequivocal that in the parkinsonian brain exogenous L-dopa does increase the concentration of striatal DA (109). However, several key issues related to the mechanisms of action of L-dopa, such as decarboxylation sites, the roles of the vesicular vs. extravesicular DA pool, phasic vs. tonic DA-mediated responses, and the contribution of different DA receptor subtypes to the therapeutic effect remain incompletely understood.

Clinical efficacy and shortcomings of long-term treatment

L-dopa is at present the most effective drug in the treatment of motor symptoms of PD (110, 111). Patients usually demonstrate a consistent clinical response to the drug. In general, bradykinesia/akinesia, rigidity, and tremor all respond to L-dopa therapy, whereas postural instability is usually unresponsive. L-dopa therapy has been associated with reduced progression of disability, morbidity, and mortality (75, 76).

In early PD, a smooth, long-duration response to L-dopa occurs, presumably due to presynaptic storage and tonic release of DA from the unaffected axons (92). In the long term, motor response oscillations and abnormal involuntary movements emerge in a significant number of patients (2). It is thought that after 5 years of L-dopa as many as 50% or more develop motor response complications (112, 113). These constitute a major therapeutic challenge.

The most common type of motor complication is the "wearing-off" or "end-of-dose" phenomenon, in which the initially stable diurnal motor state during intermittent L-dopa dosing is replaced by a progressive shortening of response to each dose. In late-stage disease, the motor response to the drug may closely correlate with its plasma levels (114).

Involuntary movements such as dyskinesia and dystonia may also emerge. One large prospective trial observed dyskinesias in one-third of the patients after approximately 1.5 years of L-dopa therapy (115). The most common form is the peak-dose dyskinesia (116), in which the peaks for plasma L-dopa and clinical effect coincide with typically choreiform movements. Dystonia presents as sustained muscle contraction leading to abnormal twisting or posturing of a body part.

The exact pathophysiology of motor fluctuations in PD is unknown. It has been suggested that the combined effects of the neurodegenerative process and long-term exposure to pulsatile plasma levels caused by intermittent L-dopa administration precipitate the emergence of motor fluctuations and dyskinesia (114, 117). Progressive loss of presynaptic dopaminergic terminals and their reduced capacity to buffer changes in L-dopa levels both in the plasma and in the brain are thought to lead to end-of-dose type fluctuations, in which tonic DA receptor stimulation is replaced by a phasic one (118, 119). Pharmacokinetic mechanisms have also been implicated (120). Involvement of other complex mechanisms (changes in postsynaptic gene expression, changes in number and sensitivity of dopaminergic/non-dopaminergic receptors) in the genesis of motor complications and dyskinesia is also under speculation.

Several attempts have been made to improve the pharmacokinetic profile of L-dopa. CR preparations (Sinemet CR, Madopar HBS) are meant to provide a more constant elevation of L-dopa plasma levels, but the bioavailability of L-dopa is reduced, and its absorption becomes even more erratic than with standard preparations (121). In theory, use of CR L-dopa might be less likely to result in development of motor complications, though no such benefit has been observable in long-term studies (122). To improve the erratic absorption of the drug, continuous duodenal infusion of L-dopa (Duodopa®) is under investigation; results have been encouraging (123).

The putative disease-modifying effect of L-dopa remains an area of controversy (114, 124). A theoretical concern is that L-dopa may promote neuronal degeneration in PD through several mechanisms (125, 126). At present, however, no conclusive evidence exists that long-term L-dopa therapy is either toxic to nigral neurons or contributory to motor complications in PD (114, 124, 127).

2.2.2. Other drugs for PD

Compounds with anticholinergic properties were the first drugs with therapeutic efficacy. Although their precise mechanisms of action are unknown (110), *anticholinergics* are thought to block central muscarinic receptors and thereby reduce striatal cholinergic preponderance. In comparison to L-dopa, the clinical efficacy of anticholinergics is modest. They appear most to benefit dystonia, rigidity, and tremor (128). Anticholinergics have undesirable adverse cognitive effects (129), thus limiting their therapeutic potential.

Schwab and co-workers were the first to report the antiparkinsonian effects of *amantadine* hydrochloride (130). Its mechanism of action in PD is likely to be complex (88, 128). Amantadine has efficacy both as monotherapy and as an adjunct to L-dopa (131). It has also been reported to reduce L-dopa-induced dyskinesia in advanced PD, possibly through its NMDA-receptor-blocking properties (132).

The central effects of DA are mediated through DA receptors, of which there are at least five types: D_1- (D_1, D_5) and D_2-like (D_2, D_3, D_4) (88, 133). *DA agonists* "mimic" the action of DA by exerting direct effects on these receptors, each having a unique affinity profile (88). The antiparkinsonian efficacy of DA agonists seems to derive mainly from stimulation of the D_2-receptor subtype, whereas D_1 stimulation has been linked to the induction of dyskinesia (134). The exact roles for D_3, D_4, and D_5 remain unknown (135).

DA agonists (bromocriptine, pergolide, gabergoline, pramipexole, ropinirole) are clinically effective both as monotherapy in early PD and as an add-on therapy to L-dopa in more advanced disease (110, 111). Their early introduction as a sole agent or as an L-dopa add-on has also been shown to delay the occurrence of motor complications, for instance, dyskinesia, in comparison to monotherapy with L-dopa (110, 112, 136, 137). DA agonists are, however, less efficacious than L-dopa and lack its clinical potency in late-

stage PD (112, 117). Therefore, L-dopa supplementation is eventually required in most patients, and only some 20% are able to remain on agonist monotherapy for 5 years (111, 112, 128).

DA agonists have been proposed to have several neuroprotective effects (117), but none have been established in clinical use.

Apomorphine is yet another DA agonist. Although not orally effective, it can be administered subcutaneously, and as such has shown efficacy in treating motor complications of long-standing PD (138).

Monoamine oxidase (MAO) catalyzes the oxidative deamination of primary amines, such as endogenous amine neurotransmitters (NA, DA), hormones (adrenaline), and exogenous dietary amines (tyramine, tryptamine). Two distinct subtypes of the enzyme exist (139). MAO-B is the subtype that predominates in the human brain and platelets (140, 141).

At daily doses of 5 to 10 mg, *selegiline* (N-propynyl-metamphetamine), a relatively selective inhibitor of MAO-B devoid of any tyramine-potentiating effect (142), has symptomatic efficacy in PD. Selegiline is able to inhibit the metabolism of DA in the brain, thereby increasing its levels both in the SN and basal ganglia (143). Other mechanisms of action such as inhibition of neuronal re-uptake of DA have also been suggested (144). Because selegiline acts irreversibly on MAO-B, the rate of recovery of enzymatic activity is slow (145).

Selegiline can extend the symptomatic effects of L-dopa and improve clinical disability in L-dopa-treated parkinsonian patients who show "wearing-off" fluctuations (146). The daily dosage of L-dopa can usually be reduced by 10 to 30%. The magnitude of improvement with selegiline is, however, modest to moderate at best, and according to some authors, its clinical benefits as add-on therapy are eventually lost (147). Selegiline has antiparkinsonian efficacy also as monotherapy (111, 148), and delays the need to initiate L-dopa (74, 148, 149).

Several neuroprotective properties for selegiline have been suggested (150, 151), but none has been proven.

As add-on therapy to L-dopa, selegiline may aggravate or induce dopaminergic adverse events (AEs): nausea, orthostatic hypotension, dyskinesia, hallucinations, confusion (146, 152). These usually respond favorably to reductions in L-dopa dosage. Selegiline monotherapy is better tolerated, but may cause dizziness, dry mouth, or insomnia. Anecdotal reports exist on the occurrence of a serotonin- or phaeochromocytoma-like syndrome in patients treated with selegiline plus either a 5-hydroxytryptamine (serotonin) uptake inhibitor or the combination of a sympathomimetic and a tricyclic antidepressant (153, 154).

Rasagiline, another irreversibly acting MAO-B inhibitor, has recently become clinically available. Like selegiline, it has shown efficacy as an L-dopa adjunct in PD patients with motor fluctuations (155).

One of the most recent additions to the pharmaceutical arsenal of PD are the inhibitors of the COMT enzyme (14, 156–158). COMT inhibitors improve L-dopa bioavailability (area under the plasma concentration time curve = AUC) by reducing its COMT-dependent metabolic loss. *Entacapone* is an inhibitor of extracerebral COMT, while *tolcapone* is able – at least to some extent – to penetrate the BBB and act also in the central nervous system. Both drugs significantly improve the clinical efficacy of L-dopa in patients with end-of-dose type motor fluctuations. COMT inhibitors, entacapone in particular, are reviewed in Section 2.3.

2.2.3. Antiparkinsonian drugs: effects on cardiovascular autonomics

Numerous reports exist on the effects of antiparkinsonian drugs on cardiovascular autonomic function in PD. The effects of L-dopa are controversial. According to many reports, acute or chronic administration of L-dopa has no discernible effect on baroreceptor reflex function (59), cardiovascular autonomic parameters (18, 30, 49, 58), hemodynamic adaptation or serum NA response to orthostatic challenge (46, 159). Significant effects have also been reported. Long-term L-dopa therapy has been shown to reduce BP in PD (160), possibly through centrally mediated mechanisms (161). Blunting of plasma NA response by dopaminergic drugs (including L-dopa) has also been suggested (69). Beneficial effects of chronic L-dopa administration on cardiovascular autonomic function, such as amelioration of heart rate (HR) response to deep breathing (162) and diminution of previously abnormal orthostatic BP fall (61), have also been reported.

Orthostatic hypotension is a widely recognized side-effect of DA agonists. It has occurred in one-third of the patients at the start of therapy, although only approximately one-third of these were symptomatic (163). Lisuride has reduced plasma NA levels, as well as blunted plasma NA and cardiovascular responses to standing up in *de novo* PD patients (164). Bromocriptine, the effects of which are probably the most extensively studied, has reduced both supine and standing systolic BP in PD, possibly through central dopaminergic or α-adrenolytic mechanisms (165). Bromocriptine has also augmented the abnormal BP fall during an orthostatic test in previously untreated patients (61).

Chronic administration of selegiline has diminished cardiovascular autonomic (especially sympathetic) responses (166) and is associated with severe orthostatic hypotension in the majority of patients, with an abolition of this effect after withdrawal of the drug (167). In untreated PD patients, an augmentation of systolic BP fall after tilting, and a blunting of systolic BP response to isometric work have occurred after 6 months on selegiline. Changes in these responses reversed to baseline after discontinuation of the drug (61).

2.3. Catechol-*O*-methyltransferase (COMT) inhibitors in treatment of PD

Inhibition of the COMT enzyme is a novel approach in the symptomatic treatment of PD. Concise reviews on the pharmacology of this enzyme and its inhibitors (7, 14, 168), and on the role of these drugs in the therapy of PD are available (157, 158, 169, 170).

2.3.1. COMT enzyme: localization and molecular structure

COMT, which catalyzes the conversion of catecholamines (adrenaline, NA, DA) to their corresponding *O*-methylated amines (4–6), is an intracellular enzyme mostly present in a soluble (S-COMT), but also in a membrane-bound (MB-COMT) form (6, 7, 14, 171).

COMT is widely distributed in mammalian tissues (14, 15): The highest COMT activity is in the liver, followed by the kidneys and the gastrointestinal tract (168, 172, 173). It is abundantly present also in the mammalian brain. In the striatum, S-COMT activity predominates in the glial cells, whereas neuronal activity mainly derives from MB-COMT (174, 175).

Mammals have a single COMT gene localized to the long arm of chromosome 22 (176, 177). This gene codes for both S- and MB-COMT, and is expressed in two transcripts of different lengths (178). Some of the extra amino acid residues present in MB-COMT

may be responsible for anchoring the enzyme into cellular membranes (179). COMT is a single-domain protein, and its active site – formed by a catalytic site of a few amino acids and the binding domain for the coenzyme *S*-adenosyl-L-methionine – is located in a groove on the enzyme surface (14, 180).

COMT catalyzes the transfer of the methyl group from *S*-adenosyl-L-methionine to one of the hydroxyl groups of the catechol substrate in the presence of Mg^{2+} (7). The two COMT isoforms differ in their substrate selectivity and enzymatic capacity: MB-COMT exhibits a markedly higher affinity for catechols than does its soluble counterpart (181) and may therefore be more important in the metabolism of catecholamines (171).

Human beings have two COMT alleles (low activity = $COMT^L$; high activity = $COMT^H$) that demonstrate autosomal co-dominant inheritance (14, 182). $COMT^L$ associates with thermolability of the enzyme (182). A single codon polymorphism in the COMT gene has been identified (183); COMT polymorphism and enzymatic activity vary between races and ethnic groups (184). Controversy has arisen over an association between $COMT^L$ homozygosity and increased susceptibility to PD (185, 186).

2.3.2. COMT enzyme: biological function

COMT and MAO are particularly important in the metabolic transformation of catecholamines (187, 188). The general role of COMT is the elimination of biologically active or toxic catecholamines and some of their hydroxylated metabolites; in various tissues, it serves as a modulator of catecholaminergic activity. MB-COMT is partially responsible for the termination of dopaminergic and noradrenergic neurotransmission, whereas the high-capacity S-COMT is mainly responsible for the elimination of biologically active or toxic (particularly exogenous) catechols and metabolites (156). In the brain, COMT may regulate the levels of DA and NA and therefore play a role in mood, behavior, and other mental processes (14, 189). In the kidneys, COMT seems to be important in the DA-mediated regulation of renal sodium excretion (190).

Metabolism of endogenous catecholamines and other substrates

Many endogenous compounds are substrates for COMT. These include amino acids with a catechol moiety (levodopa), naturally occurring catecholamines (DA, NA, adrenaline) (6), their hydroxylated metabolites, and catecholestrogens (191). However, in physiological situations, the neuronal reuptake ($uptake_1$) of DA and other catechols dominates among metabolic inactivation pathways (COMT and MAO in particular) as the most efficient clearance system for locally released catecholamines (168, 188, 192). $Uptake_1$ is also essential for the proper function of MAO in providing substrates for intraneuronal oxidative metabolism (168). *In vivo* studies with experimental animals have shown that COMT inhibition has no discernible effect on clearance of NA, adrenaline, or DA, and also that the contribution by $uptake_1$ to removal of circulating catecholamines depends on whether the systemic or pulmonary circulation is considered (193). Accordingly, inhibition of either MAO or COMT alone has little if any effect on the removal of these catecholamines on their passage through the systemic and pulmonary circulation. Although the combined inhibition of both of these enzymes has been highly effective in reducing the pulmonary clearance of NA and DA, only minor decreases have occurred in the total-body clearance of all three catecholamines (194). The metabolic disposition of catecholamines is illustrated in Fig. 1.

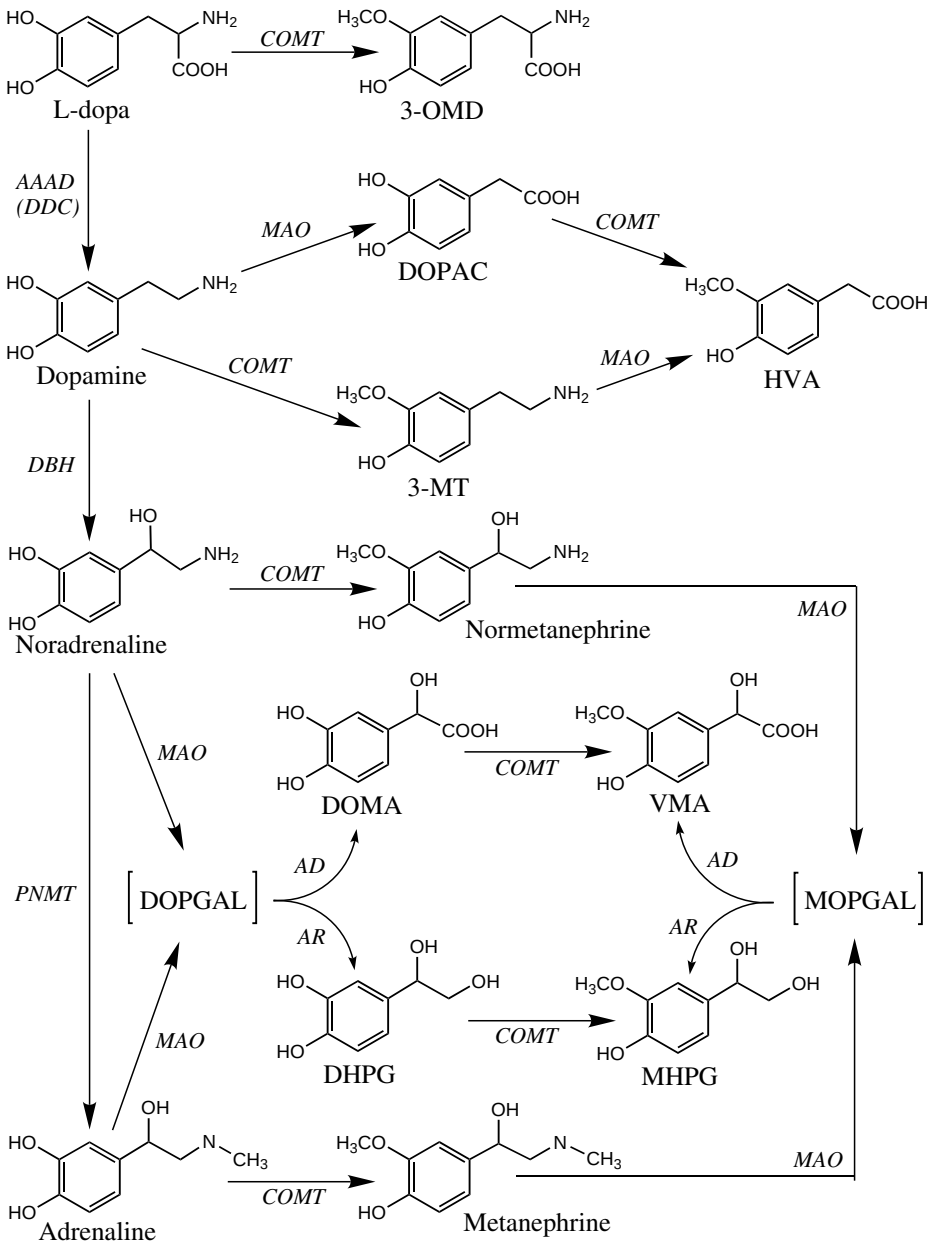


Fig. 1. Synthesis and metabolic disposition of catecholamines. Enzymes: AAAD/DDC, aromatic L-amino acid decarboxylase = dopa decarboxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine-N-methyltransferase; COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; AD, aldehyde dehydrogenase; AR, aldehyde reductase. Compounds: 3-OMD, 3-O-methyldopa; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; HVA, 3-methoxy-4-hydroxyphenylacetic acid; DOMA, 3,4-dihydroxymandelic acid; VMA, 3-methoxy-4-hydroxymandelic acid; DHPG, 3,4-dihydroxyphenylethylene glycol; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol. Metabolic intermediates []: DOPGAL, 3,4-dihydroxyphenylglycoaldehyde; MOPGAL, 3-methoxy-4-hydroxyphenylglycoaldehyde. Most catecholamines and their metabolites may also be conjugated to either sulfates or glucuronides.

Effects on L-dopa and other exogenous substrates

COMT serves as a protective barrier against detrimental effects of xenobiotics (14). Its exogenous substrates include dietary and medicinal compounds, for instance, ascorbic acid, flavonoids, and several catechol-structured drugs such as L-dopa, dobutamine, isoprenaline, benserazide, carbidopa, apomorphine, and L-threo-DOPS.

Although in physiological circumstances COMT apparently does not contribute greatly to the clearance of endogenous catecholamines, it does play a definite role in the removal of some exogenous catechol-structured drugs like isoprenaline (193). Likewise, COMT plays a major role in the metabolism and fate of exogenously administered L-dopa. In the periphery, L-dopa is metabolized mainly by two enzymatic pathways working in parallel: DDC-catalyzed decarboxylation to DA, and COMT-mediated O-methylation to 3-*O*-methyldopa (3-OMD). During L-dopa therapy, the pharmacokinetic advantages of concomitant peripheral DDC inhibition are therefore partially offset by a shift in a major portion of L-dopa metabolism towards the COMT pathway (168, 195, 196). The major metabolite 3-OMD (196, 197) is formed in proportion to the administered dose of L-dopa (198). 3-OMD has a long half-life of approximately 15 hours (199), but no intrinsic antiparkinsonian activity (200). It has also been established that exogenous 3-OMD reduces the efficacy of L-dopa therapy in PD (201). The mechanism of this antagonism may be that, being an LNAA, 3-OMD can competitively inhibit the transport of L-dopa across the intestinal mucosa and through the BBB (202). However, 3-OMD constitutes only a minor portion (about 10%) of the total LNAAs and probably has a negligible effect on the transport/uptake of L-dopa (9, 201, 203, 204).

2.3.3. COMT inhibition: a means to modify L-dopa clearance

Therapeutic principles

The rationale of selective COMT inhibition as an adjunct therapy to L-dopa in PD, and the beneficial effects of COMT inhibitors on the pharmacokinetics of L-dopa have been extensively reviewed (9, 14, 157, 205, 206). The acknowledgment of several problems associated with L-dopa treatment has led to formulation of the following therapeutic rationale: 1) As a catechol, L-dopa is subjected to extensive degradation by DDC and COMT (Fig. 2a); its pharmacokinetic properties: extensive peripheral metabolism, short $t_{1/2}$, poor bioavailability (92), are therefore therapeutically unfavorable for providing a sustained clinical response. 2) L-dopa dose-related ("end-of-dose") motor complications emerge in many patients during chronic administration of the drug (114). 3) Although COMT activity is much lower than that of DDC, it has been supposed that competition over the substrate L-dopa may take place in the gut wall (207). When peripheral decarboxylation of L-dopa is inhibited by benserazide or carbidopa, L-dopa is predominantly *O*-methylated to 3-OMD (92) (Fig. 2b). The COMT-catalyzed pathway therefore contributes significantly to the metabolic loss of peripheral L-dopa (158, 208) (Section 2.3.2.). Combining a COMT inhibitor with L-dopa and a DDC inhibitor causes the loss of L-dopa through 3-*O*-methylation to decrease and the bioavailability of L-dopa to improve (209) (Fig. 2c). Improved bioavailability leads to improved brain entry of L-dopa and allows a decrease in the dose of L-dopa without loss in its clinical efficacy (L-dopa "sparing" effect). Furthermore, the dose interval of L-dopa can be prolonged (156).

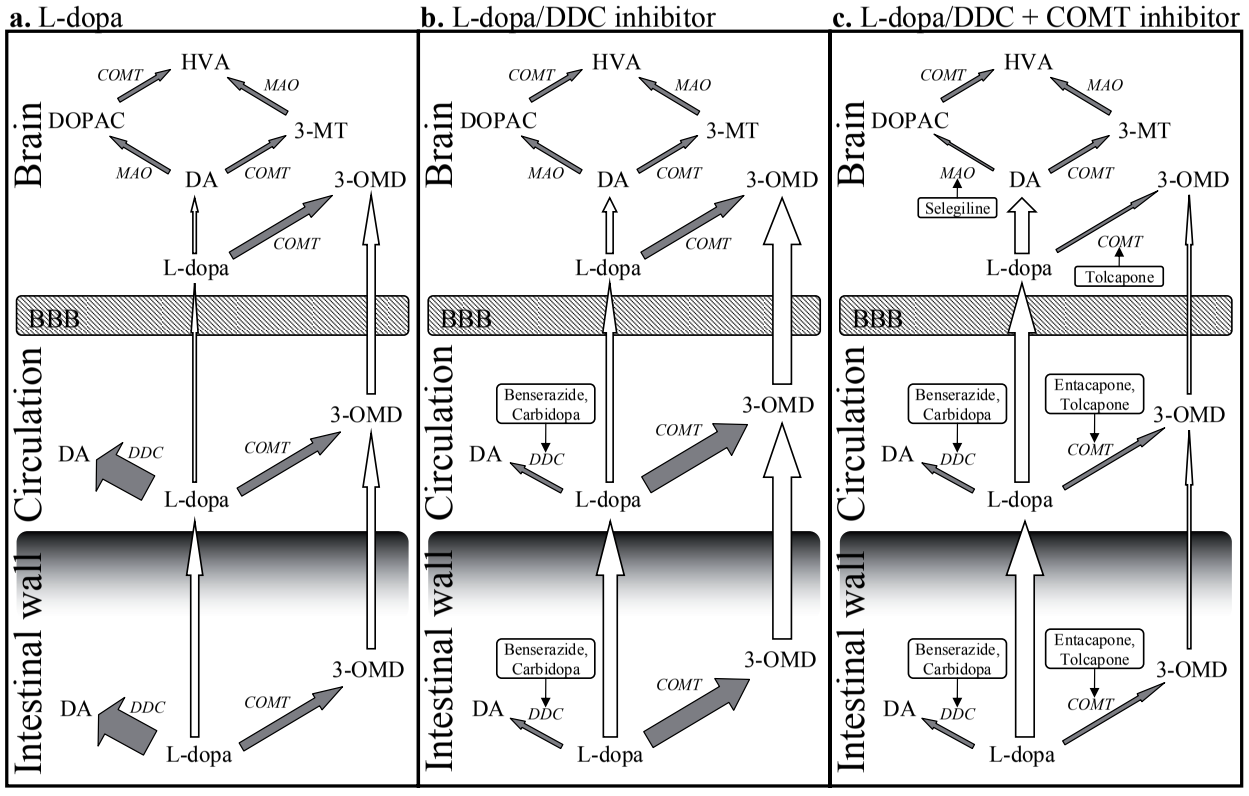


Fig. 2. The rationale of catechol-O-methyltransferase (COMT) inhibition as an adjunct therapy to L-dopa in Parkinson's disease. Effects of dopa decarboxylase (DDC), COMT, and their inhibitors on the metabolism and fate of L-dopa and dopamine (DA) shown. BBB, blood-brain-barrier; MAO, monoamine oxidase; 3-OMD, 3-O-methyldopa; DOPAC, 3–4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; HVA, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid). **a.** L-dopa without any enzyme inhibitors is extensively decarboxylated to DA in the intestinal wall and circulation. Only a minor portion of the oral dose (<5%) reaches the brain. **b.** Peripheral DDC inhibitors (benzerazide and carbidopa) improve L-dopa bioavailability and brain entry by reducing its first-pass metabolism. However, more L-dopa is lost through *O*-methylation by COMT. **c.** During simultaneous inhibition of DDC and COMT (by entacapone, tolcapone), peripheral metabolic loss of L-dopa to 3-OMD is much reduced. This results in further improvement in drug bioavailability. Entacapone does not cross the BBB, whereas tolcapone does so, at least to some extent. Selegiline inhibits MAO subtype B (MAO-B), which is predominantly located in the brain.

In the treatment of "end-of-dose" motor fluctuations, COMT inhibition has been an effective approach in providing a more prolonged maintenance of L-dopa plasma levels, more continuous dopaminergic input into the striatum, and finally a longer clinical response to the drug (169, 170).

COMT inhibitors

The first compounds found to show COMT inhibitory properties include pyrogallol and its derivatives (gallic acid, N-butyl-gallate), catechol derivatives, and tropolones (7). Some of these, such as N-butyl-gallate, demonstrate clinical efficacy in PD (210). These early compounds had qualities, however, that made them inappropriate for human use, namely lack of selectivity, low potency, and short duration of action *in vivo* (7, 14). Many of them were also rather toxic.

It has been demonstrated that catechols containing electronegative substituents such as NO_2 are potent inhibitors but poor substrates of COMT (209, 211). In the late 1980s, a class of highly potent and selective COMT inhibitors was developed. These compounds were bisubstituted catechols incorporating a nitrocatechol moiety (NO_2 in the 5'-position) as a key molecular structure (Fig. 3) (209, 212). The nitrocatechol derivatives enta-

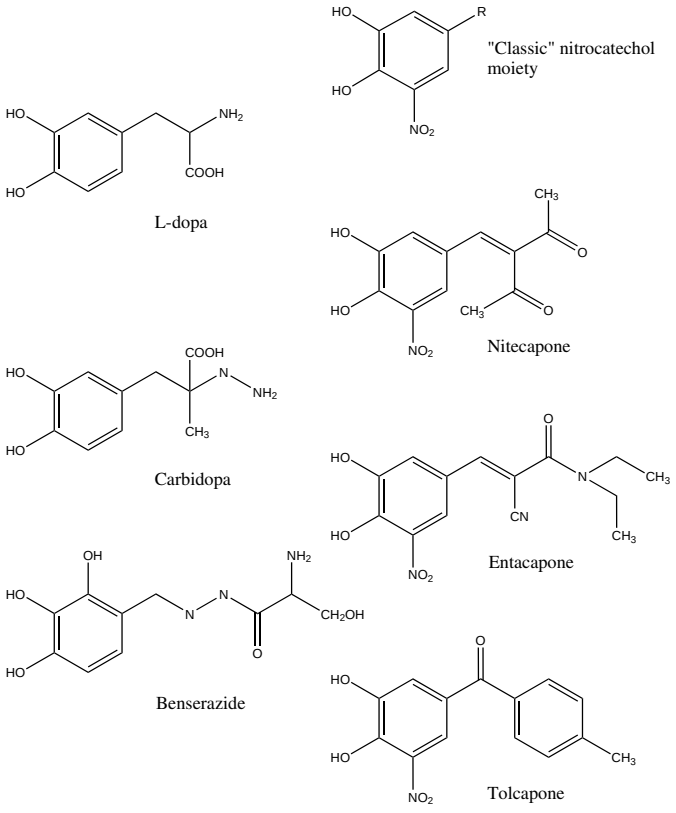


Fig. 3. Chemical structure of L-dopa, peripheral dopa decarboxylase (DDC) inhibitors carbidopa and benserazide, and some nitrocatechol-structured catechol-*O*-methyltransferase (COMT) inhibitors, with the 5'-nitrocatechol moiety, essential for highly selective COMT inhibition, shown. -R, side-chain. The aromatic side-chain of tolcapone renders it more hydrophobic than are the other two nitrocatechol compounds.

capone [OR-611; (*E*)-2-cyano-*N,N*-diethyl-3-(3,4-dihydroxy-5-nitrocinnamamide], nitecapone [OR-462; 3-(3,4-dihydroxy-5-nitro-benzylidene)-2,4-pentanedione], and tolcapone (Ro 40-7592; 4′-methyl-3,4-dihydroxy-5-nitro-benzophenone) are orally active, selective, highly potent, and reversibly acting inhibitors (156, 173, 181, 213–216). Their selectivity for COMT is much higher (by several magnitudes) than that of other catecholamine-metabolizing enzymes (212, 214-217). All of these compounds demonstrate a dose-dependent inhibitory action on the enzyme (156, 212, 215, 217). Tolcapone has more potency and a longer duration of action than do the other two drugs (14, 218, 219).

Ample data from experimental animals indicate poor BBB penetration – and therefore a principally extracerebral mode of action – for entacapone (8, 216, 218). Nitecapone demonstrates similar properties (173, 212, 217, 220). Tolcapone is able (at least to some extent) to penetrate the BBB and inhibit COMT also in the brain (8, 214, 218, 219); this has been demonstrated in humans (221). Central COMT inhibition seems, however, to be of minor significance, and the therapeutic efficacy of COMT inhibitors most probably derives from their peripheral actions only (158, 206).

Animal data are consistent with the effective COMT inhibition of each of these compounds. Markedly reduced formation of 3-OMD, dose-dependent prolongation of the $t_{1/2}$ of L-dopa, significantly improved L-dopa bioavailability, and increased levels of striatal DA have all been observed (212, 220, 222, 223). Accordingly, pre-treatment with either entacapone or tolcapone results in a substantially increased striatal uptake of [^{18}F]6-fluoro-L-dopa (an analogue of L-dopa) (224, 225).

Unilateral 6-hydroxydopamine (6-OHDA)-induced lesion of the nigrostriatal pathway is a widely used rodent model of PD (226). Dopaminergic agents such as L-dopa can induce contralateral circling behavior in 6-OHDA-lesioned rats. The COMT inhibitors entacapone, tolcapone, and nitecapone markedly potentiate this L-dopa-induced rotational behavior (220, 227), consistent with their effects on striatal DA levels.

2.3.4. COMT inhibitors: pharmacodynamic effects in humans

COMT activity

In healthy humans and patients with PD, modern COMT inhibitors show a rapid, consistent, dose-dependent, and fully reversible inhibition of erythrocyte COMT, both after single and after repeated dosing with these drugs (228–232). A 200-mg dose level of entacapone has been reported to lead to an inhibition of erythrocyte COMT activity of approximately 40% (232, 233).

L-dopa pharmacokinetics

The effects of entacapone on L-dopa pharmacokinetics and DA metabolism are summarized in Table 2. In PD patients, the $t_{1/2}$ of L-dopa is prolonged by 25 to 75%, and its AUC (bioavailability) is increased accordingly (204, 232–235). The absorption kinetics, i.e., peak plasma concentration (C_{max}) or the time to peak plasma concentration (T_{max}), of L-dopa generally remain unchanged by entacapone (Table 2) (157, 204, 232, 236, 237).

Table 2. Effects of entacapone 200 mg on the pharmacokinetics of L-dopa and dopamine metabolism in humans.

Dosing scheme, dose range (Duration of treatment)	*L-dopa AUC*	*$t_{1/2}$*	*T_{max}*	*C_{max}*	*AUC of 3-OMD*	*DOPAC*	*HVA*	*Reference*
Healthy subjects								
Single, E 50-400 mg	+42%	NC	NC	NC	-46%	+214%	NC	236
Single, LD 50-250 mg	+30 - +40%		NC - +125%	-28% - NC	-55% - -60%	+200% - +260%	-75% - NC	238
PD patients								
Single	+46%	+32%				+181%	-33%	235
Single Repeated (8 weeks)	+48% +43%	+75% +59%	NC -33%	NC	-59%			204
Single Repeated (1 week)	+38% +40%		NC	NC	NC -44%		-24% -28%	234
Single, E 50-400 mg	+23%	+39%	NC	NC	-13%	+77%	-32%	232
Single Repeated (4 weeks)	+29% +21%	NC	NC	NC	NC -45%		-21%	239
Repeated (4 weeks)	+35%	+32%	NC	NC	-63%	+201%	-17%	240
Repeated, E 100-400 mg (2 weeks)	+27%	+26%			-54%			233

AUC, area under the concentration time curve; $t_{1/2}$, elimination half-life; T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; 3-OMD, 3-*O*-methyldopa, DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; E, entacapone; LD, L-dopa; NC, not (significantly) changed

The changes in L-dopa pharmacokinetics after a single dose of entacapone remain practically unaltered when entacapone is administered repeatedly with L-dopa (204, 234, 239). During repeated, simultaneous dosing of the two drugs at 2- to 4-hour intervals, the mean daily concentration of L-dopa rose, with a 30 to 50% decrease in the peak-trough variation in its plasma levels observable (158, 204, 206). No accumulation of L-dopa plasma levels occurs, however, from one day to the next (204, 241).

In general, entacapone has similar effects on the pharmacokinetics of both CR and standard L-dopa formulations (242, 243). Unlike the case with standard L-dopa, however, entacapone seems to raise the C_{max} of CR L-dopa (243, 244).

During long-term administration of entacapone 200 mg with each dose of L-dopa, plasma levels of 3-OMD are reduced, dose-dependently (236), by some 50% (Table 2) (11, 204, 234, 239, 240).

Entacapone alters the metabolic profile of peripheral DA. Plasma levels of dihydroxyphenylacetic acid (DOPAC) become markedly increased (Table 2), indicating a shift in DA metabolism towards MAO-catalyzed degradation (Fig. 1). A moderate decrease in the AUC of homovanillic acid (HVA, the final product of DA metabolism) occurs after addition of entacapone to L-dopa therapy.

2.3.5. The role of the COMT inhibitor entacapone in treatment of PD: clinical evidence

Efficacy

Both entacapone and tolcapone are efficacious in parkinsonian patients with end-of-dose motor fluctuations (158, 169, 245).

Several factors favor the simultaneous administration of entacapone with each dose of L-dopa: First, these two drugs have pharmacokinetic similarities such as a $t_{1/2}$ of about one hour; second, the COMT inhibition by entacapone is rapidly reversible; third, a close correlation exists between plasma entacapone level and level of COMT inhibition (92, 158, 235, 236, 246). In comparison to entacapone's, both the $t_{1/2}$ and the COMT inhibitory effect of tolcapone are longer. The drug is therefore always administered in three daily doses, irrespective of L-dopa dosing frequency (157, 158, 211).

Entacapone 200 mg has been shown to be the most effective dose, both pharmacokinetically and clinically (232, 246). No further clinical benefit results from higher doses (170, 232, 247).

In early clinical studies of fluctuating PD patients, both single and repeated dosing of entacapone significantly reduced the motor disability by prolonging the duration of motor response to each dose of L-dopa (the ON-time), as measured by the UPDRS part III and other tests of motor function such as tapping and walking (204, 234, 239, 240, 247). Entacapone has lengthened the ON-time of each dose of L-dopa by 30 to 40 minutes (170), without affecting the magnitude of motor response (232, 239).

Table 3. Efficacy of entacapone in randomized, double-blind, placebo-controlled studies.

Design & Duration	*Arm*	*Patients (n)*	*ON-time[a] increase (h)*		*OFF-time[b] decrease (h)*		*Motor UPDRS change[c]*		*ADL change[c]*		*Reference*
				Δ		Δ		Δ		Δ	
Cross-over	E		2.5								240
4 weeks	P	23	0.4	2.1**	NA		NA		NA		
Parallel-group	E	103									10
6 months	P	102		~1**		~1		-2.4*		-1.1*	
Parallel-group	E	85	1.4		1.3		-3.0		-1.7		11
6 months	P	86	0.2	1.2***	0.1	1.3***	+4.2	*	-0.4	**	
Parallel-group[d],	E	129	1.7		1.6		-3.3		-1.1		248
6 months	P	74	0.9	~1*	0.9	~1*	-0.1	**	-0.2	*	
Parallel-group[d]	E	115	1.3	1.2**	1.1	*	-4.5	NS	-0.5	NS	249
6 months	P	57	0.1		0.3		-4.3		-1.1		

[a] period when mobile or capable of moving with relative ease; [b] period when immobile or incapable of moving with relative ease. [c] reduction means improvement, increase indicates worsening; [d] patients with motor fluctuations shown.

Δ, overall treatment effect (entacapone vs. placebo) ; UPDRS, Unified Parkinson's disease Rating Scale; ADL, Activities of Daily Living; E, entacapone; P, placebo. Significance levels of the treatment effect shown: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, not significant.

The relevant efficacy data from five double-blind, randomized, placebo-controlled studies (10, 11, 240, 248, 249) are summarized in Table 3. These studies corroborate evidence on the clinical efficacy of entacapone from earlier trials: The daily ON-time was significantly increased (by one hour or more), with a corresponding reduction in time spent in the "off" state (Table 4). Significant improvement was also observable in PD disability scales such as the motor and Activities of Daily Living (ADL) subscores of the UPDRS (10, 11, 248). ADL and quality of life-related parameters seem to improve also in clinically non-fluctuating patients (Table 4) (248–250).

The clinical benefits of entacapone are achieved and sustained despite a 10 to 30% reduction in the total daily dosage of L-dopa (10, 170, 251). Its clinical effects are also achieved from the first dose (204) and are sustained during long-term therapy for up to at least 3 years (10, 11, 251).

Table 4. Beneficial effects of entacapone as an L-dopa adjunct (258).

Patient group	*Effect*
L-dopa-treated, motor fluctuations	Increased ON-time[a]
	Decreased OFF-time[b]
	Reduced L-dopa dosage
	Modestly improved UPDRS motor and ADL scores
L-dopa treated, *without* motor fluctuations	Improved UPDRS ADL scores
	Improved QoL scores
	Reduced L-dopa dosage
	Improved UPDRS motor scores (some studies)
L-dopa naïve *(de novo)*	Not yet assessed

[a] period when mobile or capable of moving with relative ease; [b] period when immobile or incapable of moving with relative ease.
UPDRS, Unified Parkinson's disease Rating Scale; ADL, Activities of Daily Living; QoL, Quality of Life.

Entacapone improves the clinical response to L-dopa regardless of the type of L-dopa formulation (standard or CR), the DDC inhibitor (158, 243, 244, 248, 252), or the concomitant use of a DA agonist or selegiline or both (10, 11, 248, 249, 253).

A triple combination (TC) tablet consisting of L-dopa, carbidopa (in 4:1 ratio, respectively), and 200 mg entacapone (Stalevo™) has recently become available. TC comes in three strengths, which are bioequivalent to corresponding doses of L-dopa/carbidopa and entacapone administered separately.

No conclusive evidence is available on the efficacy of entacapone in *de novo* PD patients. It has been suggested that pulsatile stimulation of striatal DA receptors by short-acting agents (like L-dopa) may have deleterious effects by inducing motor response complications (117). Drugs that provide more continuous dopaminergic stimulation could therefore prove more beneficial (254). As COMT inhibitors can attenuate "peak-trough" variations in plasma L-dopa levels (204, 206), they might, if instituted early, be able to provide some benefits of continuous dopaminergic stimulation, meaning postponement of L-dopa-related motor complications (110, 255). Although these notions are supported

by some recent animal data (256, 257), clinical evidence is lacking. One study assessing the effects of early introduction of entacapone on emergence of L-dopa-related motor complications (STRIDE-PD) is currently underway.

Clinical safety experience

Entacapone has been well tolerated, with a good long-term safety profile, also in combined use with other antiparkinsonian agents like DA agonists, selegiline, amantadine, and anticholinergics (10, 11, 251, 259). Its discontinuation rate has not significantly differed from that during placebo (251, 259).

The clinically relevant AEs of entacapone therapy have been reviewed (170, 260), and those that occur significantly more frequently with entacapone than with placebo are listed in Table 5.

As a result of enhanced brain entry of L-dopa, entacapone may aggravate or induce dopa-related AEs, especially dyskinesia (10, 11, 248, 251, 259). This occurs almost exclusively during the first days or weeks after initiation of therapy (10, 11, 170, 248, 259), and particularly in those with a previous history of dyskinetic movements (157, 158, 170, 261). Dyskinesia can be largely controlled by reducing the total daily dosage of L-dopa (extension of dosing interval or lowering of individual doses) (170, 204, 234, 239, 248). In patients at risk (pre-existing dyskinesia, high L-dopa dosage), pre-emptive measures may be warranted, such as reducing L-dopa dosage at the time of initiation of entacapone (158, 170). Nausea usually appears soon after introduction of entacapone therapy (170, 260). Psychic dopaminergic AEs are quite rare. All dopaminergic AEs respond favorably to L-dopa dosage reductions.

Table 5. Adverse events of entacapone in long-term placebo-controlled studies, adapted from (158).

Adverse event	*Entacapone (n=806) %*	*Placebo (n=497) %*
Dopaminergic		
Dyskinesia/hyperkinesia	30.4***	17.5
Nausea	13.6***	7.4
Vomiting	3.6**	1.2
Non-dopaminergic		
Urine discoloration	10.8***	0.0
Diarrhea	10.3***	3.8
Abdominal pain	7.3*	4.2
Constipation	7.2*	4.2
Fatigue	6.1*	3.6

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (entacapone vs. placebo).

Gastrointestinal AEs (diarrhea, constipation, abdominal pain) occur significantly more frequently with entacapone than with placebo (10, 11, 170, 248, 260), but their emergence may be delayed until several weeks or months into therapy. A non-dangerous phenomenon encountered when using either entacapone or tolcapone is dark yellow/reddish-brown discoloration of the urine (10, 157, 234, 253, 262).

In contrast to entacapone, tolcapone can induce severe dyskinesia (12, 13). It also demonstrates a higher discontinuation rate due to diarrhea, which may be explosive (12).

Cases of fatal liver failure due to tolcapone therapy have numbered three (263, 264), resulting in a temporary suspension of its marketing authorization in the EU region. Its propensity to form reactive intermediates and to interfere with mitochondrial energy production has been proposed as an explanation for its hepatotoxic potential (265, 266). As for entacapone, clinical trials and post-marketing surveillance studies have demonstrated no increase in liver enzyme levels above those observed with placebo, with no treatment-related cases of acute liver failure or death (157, 158, 259, 267). During entacapone therapy, routine monitoring of liver function is unnecessary (158, 170).

Abrupt withdrawal of antiparkinsonian medication has sometimes been associated with neuroleptic malignant-like syndrome (NMS) and rhabdomyolysis (264). There is one case report of the emergence of NMS after abrupt withdrawal of tolcapone (268). So far, no such cases have been reported to occur after withdrawal of entacapone.

2.3.6. COMT inhibition and cardiovascular autonomics in PD

The high capacity of COMT has therapeutic relevance in respect to both the efficacy and safety of COMT-inhibitor treatment. It has been suggested that only a minor fraction of the capacity of the enzyme is ever needed (14), which implies that although substantial COMT inhibition with significant pharmacodynamic effects is achievable in patients with PD, the remaining COMT activity should still be capable of eliminating naturally occurring catecholamines. The level of COMT inhibition achieved by nitrocatechol compounds is far from complete. Nitecapone, as one example, is able to reduce COMT activity by 50 to 60%, but not much more despite substantial increases in its dosage (229).

Catecholamine metabolism

In theory, therapeutic inhibition of COMT could increase plasma levels of endogenous catecholamines (especially during physical exercise, when their release is markedly augmented) or of the exogenous catechol-structured compounds that are substrates of the enzyme. However, the reported effects of the nitrocatechol COMT inhibitors nitecapone and entacapone on plasma catecholamine (DA, NA, and adrenaline) levels in healthy humans have consistently shown that such an increase does not occur, and that both resting and exercise plasma levels of unconjugated (free) catecholamines remain unchanged after both single and repeated dosing of either nitecapone (up to 100 mg t.i.d.) or entacapone (up to 800 mg t.i.d.) (16, 17, 269–271). An equally consistent finding in these studies has, however, been the altered metabolic profile of catecholamines caused by these COMT inhibitors. They have been shown to reduce plasma levels of methylated (COMT-dependent) metabolites of catecholamines (156). Levels of MAO-dependent metabolites have increased accordingly. Some of the changes in catecholamine metabolism are dose-dependent (17, 242). Changes in other pathways of catecholamine metabolism, for instance conjugation, also occur (270).

In healthy volunteers, following single or multiple dosing of either nitecapone or entacapone, an altered metabolic profile of peripheral DA has been evident in significantly increased plasma levels of DOPAC (Fig. 1) and decreased urinary excretion of HVA (17, 269-271). Similar findings emerged in healthy subjects receiving single doses of either nitecapone or entacapone as adjuncts to L-dopa/carbidopa. Their plasma DOPAC levels are markedly increased (228, 236, 242), whereas those of HVA are either decreased (228,

242) or remain unchanged (236). The urinary excretion of methylated metabolites of DA (3-methoxytyramine and HVA) also decreases (228). Similar changes in catecholamine metabolism have taken place in L-dopa-treated PD patients: After either single or multiple dosing of entacapone for up to one month, plasma DOPAC levels are significantly increased, whereas plasma levels and urine excretion of HVA are somewhat decreased (232, 235, 240).

Changes also occur in the metabolic profile of NA and adrenaline. Plasma levels of 3,4-dihydroxyphenylethylene glycol (DHPG, a MAO-dependent metabolite of NA and adrenaline, Fig. 1) have been markedly higher, and those of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG, a COMT-dependent metabolite) lower after single or multiple dosing of either nitecapone (269, 270) or entacapone (16, 17, 271, 272). These changes have been suggested to be due to both shunting of the metabolism towards MAO-catalyzed oxidation and, in the case of DHPG, for instance, inhibition of COMT-catalyzed methylation in the subsequent steps of the metabolic pathway (Fig. 1) (16). After nitecapone, changes in the profile of urinary catechol metabolites, such as the reduced urinary excretion of 3-methoxy-4-hydroxymandelic acid and metanephrine have also occurred (228, 269). COMT inhibition seems to elevate plasma levels of some conjugated catecholamines. After repeated administration of nitecapone, a three-fold increase in the plasma concentration of conjugated adrenaline has occurred (270).

Data are less concerning the effects of tolcapone on plasma catecholamines and their metabolites. Similar to the other two nitrocatechol COMT inhibitors, tolcapone as an adjunct to L-dopa has raised plasma DOPAC levels, and reduced those of HVA (230, 273). Effects of tolcapone on plasma HVA are more pronounced than are those of entacapone, probably due to its central COMT inhibitory action. Prolonged therapy in parkinsonian patients with tolcapone as an adjunct to L-dopa also significantly elevates plasma levels of the actual catecholamines DA, NA, and adrenaline (273, 274).

In addition to the endogenous catecholamines, several catechol-structured drugs (adrenaline, isoprenaline, dobutamine, apomorphine) are substrates of COMT. In experimental animal models, COMT inhibition potentiates the cardiovascular effects of exogenously administered catecholamines (275, 276). Against this background, a theoretical safety concern arises regarding possible drug-drug interactions between these agents. In one study, a single 400-mg dose of entacapone in healthy subjects did not significantly change plasma concentrations of intravenously administered adrenaline or isoprenaline (277). Systemic clearances of these two catecholamines by either the intact fraction of COMT activity or other metabolic routes are likely explanations for these findings.

Another concern is potential interaction between COMT inhibitors and other drugs having an effect on catecholamine turnover. In healthy humans, resting and exercise plasma levels of neither NA nor adrenaline were affected by the simultaneous inhibition of COMT by entacapone and of neuronal re-uptake by imipramine (272). Similar findings have resulted from co-administration of desipramine and tolcapone (278).

Hemodynamics and cardiac rhythm

Clinical evidence has, thus far, consistently shown that single or multiple administration of either nitecapone (up to 100 mg t.i.d.) or entacapone (up to 800 mg t.i.d.) to healthy subjects does not change rest/exercise hemodynamics or cardiac rhythm (16, 17, 269-272). In L-dopa-treated patients with PD, resting hemodynamics has not been affected by single or multiple dosing of entacapone (232, 234, 235). Although a more pronounced orthostatic drop in systolic BP has occurred after entacapone than after placebo (240), this has been clinically non-significant, and in parkinsonian patients, orthostatic hypoten-

sion itself has seldom been a problem during entacapone therapy (170, 232, 240). In phase III studies, manifestations of orthostatic hypotension such as dizziness and falls have been equally frequent in the entacapone and placebo arms, with no differences in the supine and standing BP, HR, or ECG (10, 11, 248, 259).

Pharmacodynamic interactions between COMT inhibitors and some vaso-/neuroactive drugs influencing hemodynamics and cardiac rhythm have also been investigated in healthy human subjects. Pressor response to intravenously administered tyramine has not been enhanced by nitecapone (279). Similarly, entacapone does not significantly change BP response to either isoprenaline or adrenaline infusions (277), but the chronotropic effect of isoprenaline is potentiated, with caution advised in considering the use of entacapone with any exogenous catecholamines that are substrates of COMT (277).

Hemodynamic effects of COMT inhibition have also been studied in a subset of MSA patients with profound autonomic involvement. In contrast to PD, entacapone dose-dependently increases the systolic BP in MSA patients (without any changes in plasma levels of DA, NA, or adrenaline) (280). In addition, the systolic BP response to phenylephrine during COMT inhibition was ten-fold higher than that of healthy controls. One suggestion is a particular susceptibility of MSA patients to the hemodynamic effects of COMT inhibition due to their impaired baroreflex function and vascular NA hypersensitivity in contrast to that of healthy controls and PD patients with intact autonomic function. An additional hypothesis is the predominant effect of COMT inhibition on synaptic rather than on circulating NA turnover.

No interactions related to hemodynamics or cardiac rhythm were reported in healthy humans during combined use of entacapone plus imipramine (272) or tolcapone plus desipramine in conjunction with L-dopa/carbidopa (278). The chronotropic effect of these re-uptake inhibitors is not enhanced by concomitant COMT inhibition. Based on the clinical data available, no clear hemodynamic interaction between entacapone and either tricyclic antidepressants or serotonin uptake inhibitors has occurred in patients with PD (267).

Parameters of cardiovascular autonomic function

Few studies with small numbers of patients implement a rigorous assessment of the effects of COMT inhibitors on cardiovascular autonomic responses in PD. In one open randomized, cross-over study with eight patients, cardiovascular autonomic responses to sympathetic or parasympathetic stimuli were unchanged by a single dose of entacapone 200 mg given as an adjunct to L-dopa (235). Parameters of HR variation determined from 24-hour ambulatory ECG recordings were not changed after 6-month therapy with tolcapone in conjunction with L-dopa in seven patients with PD (281).

Co-inhibition of COMT and MAO enzymes

Anecdotal reports are available on clinically significant drug-drug interactions between selegiline and some catecholamines and their uptake inhibitors (Section 2.2.2.). Cardiovascular safety aspects of the simultaneous inhibition of MAO-B and COMT enzymes have not yet been studied much in humans. In theory, for several reasons co-inhibition of these two enzymes should not compromise cardiovascular function: First, neuronal re-uptake is the principal peripheral route of inactivation of endogenous catecholamines, and metabolic pathways like COMT and MAO play only a supplementary role: Second, the peripheral COMT pool is only partially inhibited by these nitrocatechol compounds, leaving substantial enzymatic activity intact: Third, at the dosages used in the treatment

of PD, selegiline is highly MAO-B selective. Because this subtype of MAO is almost exclusively localized in the central nervous system, selegiline should not contribute to the turnover of peripheral catecholamines.

According to one report involving PD patients chronically treated with selegiline and L-dopa, acute administration of tolcapone up to doses of 800 mg was well tolerated, with no cardiovascular adverse effects (282). The effects of simultaneous inhibition of COMT by entacapone and MAO subtype A (MAO-A) by moclobemide has been investigated in healthy subjects, both at rest and during enhanced catecholamine release (exercise). In contrast to MAO-B, MAO-A predominates in the peripheral tissues. However, the combined use of entacapone and moclobemide in single therapeutic doses has no effect on plasma levels of free catecholamines, BP, HR, or ECG, when compared to the use of either drug alone, or to the use of placebo (271).

3. AIMS OF THE STUDY

The main purpose of the present study was to evaluate – in a double-blind, placebo-controlled setting – the safety profile of the COMT inhibitor entacapone after repeated dosing with the drug as an L-dopa adjunct to patients with PD. The more specific aims were:

1. Evaluation of the cardiovascular safety of entacapone
    - during simultaneous administration with selegiline by using repeated measurements of hemodynamics, ECG, and plasma catecholamine levels (I, II)
    - by investigating its effects on cardiovascular autonomic function (III)
    - by studying its effects on cardiorespiratory exercise performance (IV)
2. Evaluation of the clinical efficacy and tolerability of the simultaneous administration of entacapone and selegiline by use of repeated assessments of motor disability, dyskinesia, and ambulatory motor activity (I, II).

4. SUBJECTS AND METHODS

4.1. Subjects

A total of 39 patients with idiopathic PD were investigated in three clinical studies. The characteristics of their disease met the main clinical diagnostic criteria of the United Kingdom PD Society Brain Bank (81). Characteristics of the patients are summarized in Table 6.

The clinical severity of PD in the study population was either mild or moderate (stages I–III) according to modified H&Y staging (79). The patients used L-dopa as their main antiparkinsonian therapy and were all L-dopa responders. The major inclusion and exclusion criteria and distribution of individual patients are presented in Tables 7 and 8.

Table 6. Characteristics of 39 Parkinson's disease patients in the studies of entacapone.

	Study No. (Publication)		
Characteristics	*First (I)*	*Second (II)*	*Third (III & IV)*
Number of subjects	13	16	15
Women / Men	6 / 7	4 / 12	4 / 11
Age (years)[1]	65 ± 8	63 ± 8	57 ± 8
Weight (kg)[1]	NA	71 ± 11	74 ± 10
Height (m)[1]	NA	1.69 ± 0.09	1.71 ± 0.10
Duration of PD (years)[1]	5.5 ± 5.2	4.6 ± 4.4	5.8 ± 3.4
Duration of L-dopa therapy (years)[1]	4.4 ± 3.3	3.8 ± 3.3	5.3 ± 2.6
Duration of fluctuations (years)[1]	2.2 ± 2.0	1.9 ± 2.4	2.8 ± 1.5[3]
Total daily L-dopa dose (mg)[1]	723 ± 101	722 ± 160	720 ± 160
Hoehn & Yahr (n)[2]			
Stage 1	–	5	1
Stage 1.5	–	–	3
Stage 2	10	10	10
Stage 2.5	–	–	1
Stage 3	3	1	–

[1] Mean ± SD (when applicable); [2] Hoehn & Yahr stage determined during ON-state; [3] Includes those with motor fluctuations (N=12); PD, Parkinson's disease; NA, not available.

Table 7. Major inclusion and exclusion criteria in studies 1–3.

Inclusion criteria	*Applies to study*
Clinically mild-moderate Parkinson's disease (Hoehn & Yahr stages I–III)	All
End-of-dose ("wearing-off") -type motor response fluctuations	1,2
Either stable or fluctuating response to L-dopa	3
Standard- (immediate-) release L-dopa/DDC inhibitor	1,2
Standard- (immediate-) and/or controlled release L-dopa/DDC inhibitor	3
Stable daily dosage of L-dopa for ≥1 month	All
Stable (for ≥1 month) dosage of a dopamine agonist, amantadine, or anticholinergic, if used	All
Exclusion criteria	
Clinically severe Parkinson's disease (Hoehn & Yahr stages IV–V)	All
Stable motor response to L-dopa ("non-fluctuator")	1,2
Marked/disabling on-period dyskinesia	All
Random ("on-off") motor response fluctuations	All
Clinically significant and/or unstable co-morbid (e.g., cardiovascular, pulmonary, renal, hepatic, psychiatric) state	All
Use of selegiline (<1 month prior entering the study)	1,2
Recent (<1 month) use of dopamine receptor blocking agents (e.g. neuroleptics and their anti-emetic derivatives)	All
Recent (<1 month) use of monoamine-oxidase (MAO) inhibitors (either non-selective or MAO-A selective)	All
Recent (<1 month) use of tricyclic antidepressants (e.g., amitriptyline)	1
Use of sympathomimetics (α-/β-receptor agonists)	All
Use of certain antiarrhythmic/-hypertensive drugs (e.g., digitalis, ACE-inhibitors, Ca-channel blockers, α-/β-receptor antagonists)	All

DDC, dopa decarboxylase; ACE, angiotensin converting enzyme.

Before entering either the first or second study, any patient using CR L-dopa was switched at the time of recruitment to approximately equivalent dosage of a standard-release preparation. This new dosage was then gradually adjusted to achieve satisfactory clinical benefit. Upon entering the first study, all patients were taking L-dopa/benserazide t.i.d or q.i.d. (mean = 3.6 doses/day). In the second study, either L-dopa/benserazide (n=13) or L-dopa/carbidopa (n=3) was used in 3 to 5 daily doses (mean = 3.4 doses/day). In the third study, patients used standard-release (n=5), CR (n=4), or both (n=6) preparations in 3 to 5 daily doses (mean = 3.3 doses/day). However, CR was taken only at night. Although dosages of all antiparkinsonian drugs were to remain unchanged throughout the studies, clinically indicated (such as for marked dyskinesia) adjustments in L-dopa dosage were allowable.

In general, no drugs that could adversely interfere with either the conduct or the assessment of responses in the studies (DA receptor-antagonists, antiarrhythmic drugs) were allowed (Table 7), and use of such drugs was prohibited throughout the course of the studies.

Table 8. Distribution of the patients with Parkinson's disease in studies 1–3.

Subject No.	*Study No.*	*Age (years) / Gender*	*Weight (kg) / Height (cm)*	*Duration of*			*H&Y stage*	*Dopamine agonist (mg)*
				PD (years)	*L-dopa therapy (years)*	*Fluctuations (years)*[1]		
1	1,2	72 / M	62 / 172	11	10	10	3	
2	1	45 / F	NA	8	5	3	2	
3	1	75 / F	NA	1	1	1	2	
4	1	71 / F	NA	1	1	1	2	
5	1	65 / F	NA	4	4	1	2	
6	1	76 / F	NA	2	1	1	2	
7	1	68 / M	NA	2	2	1	2	
8	1,2	58 / M	70 / 182	3	3	1	2	Pergolide (3)
9	1,2	71 / M	74 / 172	7	7	5	2	
10	1	65 / F	NA	6	6	4	3	
11	1,2	58 / M	73 / 170	3	3	1	2	
12	1	62 / M	NA	7	5	3	2	
13	1	66 / M	NA	20	12	1	3	Bromocriptine (20)
14	2	57 / M	54 / 165	1	1	1	1.5	
15	2	58 / M	72 / 173	13	11	2	3	
16	2	73 / F	57 / 158	1	1	1	2	
17	2,3	62 / M	81 / 175	5	5	2	2	
18	2	68 / F	52 / 152	2	2	1	2	
19	2	56 / M	93 / 176	5	4	0	2	
20	2	73 / F	66 / 157	5	4	1	2	
21	2	66 / F	74 / 156	2	1	1	1	
22	2	47 / M	75 / 176	1	1	1	1	
23	2	59 / M	82 / 176	1	1	1	1.5	
24	2	68 / M	67 / 175	14	7	2	1.5	Bromocriptine (30)
25	2	60 / M	80 / 175	1	1	1	2	
26	3	43 / F	63 / 167	6	6	2	2	Pergolide (1.5)
27	3	56 / M	74 / 165	8	8	6	2	
28	3	60 / F	71 / 166	8	7	NF	1.5	Bromocriptine (15)
29	3	60 / F	58 / 154	9	8	NF	2	
30	3	56 / M	73 / 164	1	1	NF	1.5	
31	3	46 / M	90 / 183	6	6	4	2	Bromocriptine (30)
32	3	48 / M	70 / 171	3	3	2	2	Bromocriptine (30)
33	3	60 / M	71 / 173	2	1	1	1	
34	3	61 / M	67 / 171	2	2	1	2	Bromocriptine (15)
35	3	49 / M	87 / 186	5	5	2	2	Bromocriptine (20)
36	3	69 / M	79 / 168	6	6	3	2.5	
37	3	47 / M	82 / 178	5	5	5	2	
38	3	65 / F	60 / 156	6	6	2	1.5	
39	3	63 / M	85 / 185	15	10	3	2	

In the first and second studies selegiline was prohibited for one month before and throughout the study, whereas in the third study it was used by every patient in doses of either 5 (n=4) or 10 (n=11) mg o.d. The total daily doses for dopamine agonists are given. None of the patients were on amantadine or anticholinergic therapy. PD, Parkinson's disease; H&Y, Hoehn & Yahr; F, female; M, male; NA, not available; NF, non-fluctuator; [1]end-of-dose (wearing-off) type fluctuations.

4.2. Methods

4.2.1. Design of the studies

Treatment comparisons and course of the studies

Each study was approved by the Ethics Committee of the Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland, and conducted in accordance with the guidelines of the amended Declaration of Helsinki. After being provided with oral and written information on the study objectives, design, and possible risks and discomforts involved, the patients gave their written informed consent.

All the studies were preceded by similar screening procedures, which included a general physical examination, assessment of supine/standing BP and HR (Section 4.2.2.), ECG (Section 4.2.2.), sampling of venous blood and urine for laboratory safety tests (Section 4.2.6.), and assessment of motor disability (Section 4.2.7.).

In the third study, screening also included determination of forced vital capacity (FVC), forced expiratory volume during one second (FEV_1), FEV% (=FEV_1/FVC), peak expiratory flow, maximal expiratory flow at 25% and at 50% of FVC, and maximal mid-expiratory flow by use of flow-volume spirometry, and comparison of results with the reference values of Viljanen (283). Direct maximal voluntary ventilation was also calculated. The results of all screening investigations were available before patients were enrolled into the studies.

All three studies were of double-blind, placebo-controlled, crossover design (Fig. 4), and patients were randomly allocated to study treatments (Table 9). The randomization procedure was carried out by the Unit of Biostatistics and Data Management, Orion Pharma, Espoo, Finland, using specific software.

Table 9. Treatments contrasted in the three studies.

Study	*Comparators*	Fixed treatments
First	a) Selegiline 10 mg o.d.	Entacapone 200 mg with each dose of L-dopa/DDC inhibitor (3–4 daily doses)
	b) Placebo o.d.	
Second	a) Selegiline 10 mg o.d.	L-dopa/DDC inhibitor (3–4 daily doses)
	b) Entacapone 200 mg (× 3–4)	
	c) Selegiline 10 mg o.d. + Entacapone 200 mg (× 3–4)	
Third	a) Entacapone 200 mg (× 3–5)	L-dopa/DDC inhibitor (3–5 daily doses)
	b) Placebo (× 3–5)	

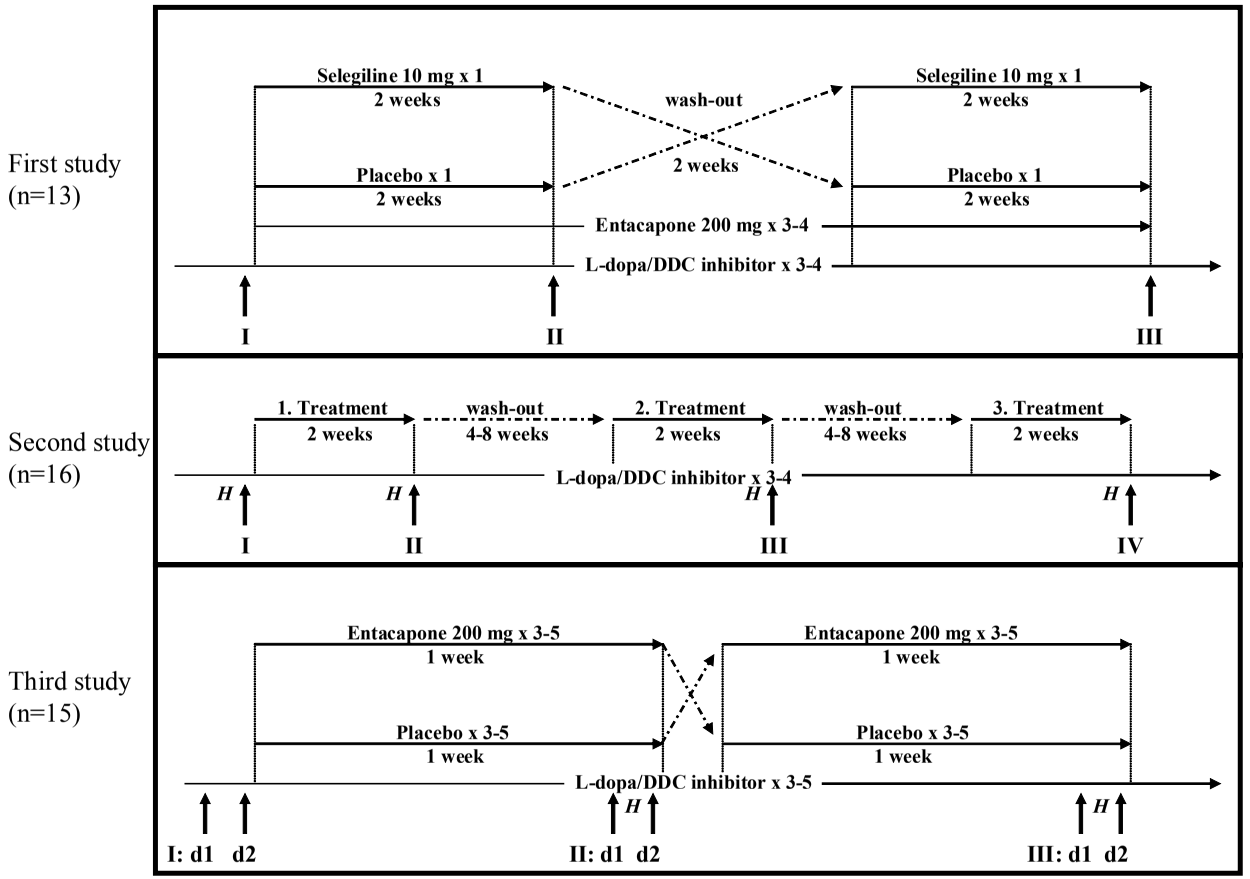


Fig. 4. Study flow-charts. All were randomized, double-blind, placebo-controlled studies with a crossover design. In the second study, the patients were allocated to receive three treatments (entacapone 200 mg × 3–4 plus placebo pro selegiline × 1, placebo pro entacapone × 3–4 plus selegiline 10 mg × 1, entacapone 200 mg × 3–4 plus selegiline 10 mg × 1) in random order as adjuncts to L-dopa. Thick vertical arrows indicate the study visits. d1 & d2, day 1 and 2, respectively; *H*, ambulatory (Holter) ECG.

The duration of each treatment period was either one (third study) or 2 weeks (first and second study). The first study included a 2-week washout (no selegiline) between treatments; in the second, there were two 4- to 8-week washouts (no entacapone or selegiline), one between each two treatment periods (Fig. 4). The third study included no washouts.

Course of the study visits

The main assessments were conducted during *study visits* (Fig. 4). Within each study, the general course of all study visits was identical. The first (control) visit was held prior to any study treatment, serving as a baseline, open-label assessment of L-dopa response in the first and second studies (Section 4.2.7.). In the third study, assessments during the control visit were performed after overnight withdrawal of L-dopa (practical off-state). During the remaining study visits the assessments were done in a timely manner after the administration of the study treatments with the morning dose of L-dopa. The study visits were conducted at the Outpatient Department of Neurology, Helsinki University Central Hospital, Helsinki (first and second study), whereas the function tests of cardiovascular autonomics (Section 4.2.3.) and the exercise tests (Section 4.2.4.) of the third study were performed in the Laboratory of Clinical Physiology and Nuclear Medicine, Helsinki University Central Hospital, Helsinki.

Study visits were each preceded by a 24-hour ambulatory (Holter) ECG (second and third study, Section 4.2.2.) and activity (actigraphy, first and second study, Section 4.2.8.) recordings.

The course of the study visits is described in Table 10. The patients refrained from taking their morning antiparkinsonian medication at home before arriving at the hospital. After arrival, the Holter (second study) and Actigraph (first and second study) devices were detached, and an intravenous catheter was inserted in the antecubital vein for blood sampling (first study).

After baseline assessments of the first and second studies, an *L-dopa test* was carried out. Appropriate study drugs were administered at 8 a.m., and the patients were then followed for 6 hours. The follow-up included repeated assessments of clinical (Section 4.2.7.) and hemodynamic (BP and HR) parameters (Table 10). In the first and second studies, the patients also self-evaluated their sleep, using a specific sleep questionnaire (Section 4.2.8.) supplementary to the ambulatory activity monitoring. The next doses of antiparkinsonian drugs were allowed at 2 p.m., the time of their departure from the hospital.

Table 10. Course of the study visits.

	Time: h	8		9		10		11		12		13		14
	min	*bl*	.30		.30		.30		.30		.30		.30	
	Study													
DRUG INTAKE	1,2	X												
- " -	3			X[a]				X[b]						
ASSESSMENTS (Section)														
Biochemistry (4.2.6.)														
Blood & urine safety	1,2	X												
- " -	3									X[c]				
L-dopa & entacapone PK	1	X	X	X		X		X		X		X		X
3-OMD	3									X[c]				
Catecholamines	1	X				X								
Plasma noradrenaline	3									X[c]			X[c]	
S-COMT activity	1			X[c]										
MAO-B activity	1					X								
Cardiovascular & respiratory														
Supine/standing BP & HR (4.2.2.)	1,2	X		X		X		X		X		X		X
ECG (4.2.2.)	1,2	X			X									
ECG (4.2.4.)	3									X[b]			X[b]	
Cardiovascular autonomics[a] (4.2.3.)	3					X----------					----X			
Cardiorespiratory exercise[b] (4.2.4.)	3											X----------		X
Maximal airway pressures (4.2.5.)	3									X[b]				
Clinical														
Modified motor UPDRS (4.2.7.)	1,2	X[d]	X	X	X	X	X	X	X	X	X	X	X	X
- " -	3									X[b]			X[b]	
AIMS (4.2.7.)	2	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse event questioning (4.2.9.)	1,2	X												X
- " -	3			X[a]				X[b]					X[b]	

bl, baseline assessments (before drugs); PK, pharmacokinetics; 3-OMD, 3-O-methyldopa; S-COMT, soluble catechol-*O*-methyltransferase; MAO-B, monoamine oxidase type B; BP, blood pressure; HR, heart rate; UPDRS, Unified Parkinson's Disease Rating Scale; AIMS, Abnormal Involuntary Movement Scale. [a]day 1 of study visits; [b]day 2 of study visits; [c]not assessed during control visit; [d]scored twice in study 1.

The study visit schedule of the third study was rather different (Table 10). It included 2 consecutive days of assessment with an ambulatory ECG registration (Holter) in between. On day 1, the patients arrived at 8:45 a.m., and the study medication (omitted from the control visit) was administered at 9 a.m. On day 2, the patients arrived at 10:45 a.m. The Holter device was detached, and the study drugs were administered at 11 a.m. (but not on the control visit). On either day 1 or 2, the patients were forbidden to take dopaminergic drugs other than the study medication (DA agonists, selegiline) prior to the tests. On day 1, tests of cardiovascular autonomic function (Section 4.2.3.) were performed, followed by a maximal work-conducted exercise test (Section 4.2.4) on day 2.

Within the first and second studies were also several short *safety visits* to the Outpatient Department (see original publications for details). These occurred during each treatment period shortly after initiation of study therapy, during washouts, and post-study. During safety visits, a modified motor UPDRS was rated, and supine/standing BP and HR, ECG, AEs, and (during certain visits) blood safety parameters were assessed. The investigators were also entitled to make adjustments to the L-dopa dosage, if clinically indicated.

4.2.2. Assessment of cardiac rhythm and hemodynamics

Responses to L-dopa test: blood pressure, heart rate, ECG

Both the schedule and the methods for assessment of hemodynamic variables and ECG were similar in the first and second studies. Supine and standing BP and HR were measured after 5 minutes of rest and 3 minutes of standing (bedside orthostatic test), respectively, first before drug intake (baseline) and then once per hour for 6 hours following administration of study drugs. The device used in the measurements (Omron BP monitor HEM 706) was calibrated at Orion Pharma, Espoo, Finland. It applied an oscilloscopic method of assessment and gave a digital read-out of the variables. Mean daily values for both supine and standing systolic BP, diastolic BP, and HR during each study visit were calculated from the consecutive measurements (n=7) of each visit. Changes in systolic BP, diastolic BP, and HR during each orthostatic test were calculated by subtracting supine values from values during standing. Mean daily changes in these variables were calculated as the mean of the differences (n=7) between standing and supine values during each study visit.

ECG was recorded before drug intake at 8 a.m. (baseline) and then at 9:30 a.m. (+1.5 hours from drug intake) in both studies. The recording was performed at rest from 12 standardized leads (bipolar limb I, II and III; unipolar limb aVR, aVL and aVF; unipolar chest V_1–V_6) using self-adhesive electrodes. The method (recording device manufactured by Siemens) included automated procedures for calibration before each recording and a computer-assisted analysis of rate, rhythm, and conduction times (PQ and QT intervals and QRS duration). However, all these variables were also determined manually (by J.L.) from the ECG printouts.

Ambulatory ECG

All visits (second study) and day 2 of the second and third visits (third study) were preceded by continuous 24-hour ambulatory ECG (Holter) recording (284). For that purpose, the patients arrived at the Outpatient Department on the previous mornings for the mounting of the device (Marquette®). The registration was performed by directly recording the 2-channel ECG signal through bipolar leads using self-adhesive skin electrodes (four recording, one ground) placed in inferior- and V_5-like positions. The waist-worn recording unit was programmed for automatic calibration, and the data was stored in standard C-cassettes. The patients were instructed to keep a log of the quality and timing of any symptoms experienced during recording and also to press a specific event-trigger on the device whenever such a symptom would occur. The device was detached, and the patient logs were collected on the following morning upon their arrival at the Outpatient Department.

Analysis of the recordings was carried out by a qualified cardiologist (Dr. J. Partanen) from the Department of Medicine, Helsinki University Central Hospital, using a semi-automated ambulatory ECG analysis system manufactured by Marquette. Variables of interest were the occurrence of supraventricular extrasystoles (SVES, beats/hour), ventricular extrasystoles (VES, beats/hour), ventricular tachycardias (number of runs per recording), and also the mean HR during recording (HR_{mean}, beats/min).

4.2.3. Cardiovascular autonomic responses

During each study visit in the third study, patients were subjected to cardiovascular reflex testing initiated 60 min after administration of the study drugs. This test was performed in a sound- and temperature-controlled (ambient temperature a constant 24°C) environment at the Laboratory of Clinical Physiology. A battery of four tests of cardiovascular autonomic function, all based on measuring of BP and HR responses to appropriate stimuli, was performed according to established methods (47, 285). The tests were a deep breathing test, an orthostatic test, Valsalva's maneuver, and a sustained (isometric) handgrip test. These tests are considered to be simple to perform in a clinical setting (286).

The primary method of assessment in the first three tests was to measure HR variation (HRV) in response to maneuvers that change vagal (parasympathetic) or sympathetic outflow to the heart or both. In practice, this was accomplished by manually measuring R-R interval variation from 12-lead ECG strips recorded during the tests. BP responses – which additionally reflect sympathetic outflow to blood vessels – in the orthostatic and hand-grip tests were recorded manually with a non-invasive intermittent cuff and aneroid manometer, and with auscultation of Korotkoff sounds I and IV.

In addition to statistical comparison of continuous variables from each test (Section 4.3.2.), responses were dichotomously categorized as either normal or pathologic, based on age-related reference values from a healthy Finnish population (287). In the evaluation of orthostatic hypotension, consensus criteria for its definition were either a ≥20 mmHg decrease in systolic or ≥10 mmHg decrease in diastolic BP or both during 3 minutes of standing (288).

Deep breathing test

In the first test of cardiovascular autonomic function, HRV was studied in response to deep breathing. A 12-lead ECG was continuously recorded while the patients, lying supine, were instructed to take deep breaths at a rate of six breaths/min (10 seconds per respiratory cycle), a rate found to produce maximum HRV (289). A study nurse used verbal cues to pace the effort. The shortest (inspiratory) and longest (expiratory) R-R intervals corresponding to six consecutive respiratory cycles were manually measured (by J.L.) from the ECG tracings. Values for R-R interval (mm) were transformed to HR (beats/min) by the formula HR = 1500 / RR interval. The mean of differences was calculated between maximum and minimum HR in each cycle (*deep breathing difference*).

Orthostatic test

In the second test of autonomic function, cardiovascular responses to active standing were studied in a 3-minute orthostatic test. BP was first measured and ECG recorded after 10 minutes of quiet, supine rest. The patients were then asked to stand up abruptly from a supine position. During the 3 minutes of standing, with feet placed slightly apart and without external support, ECG tracings were recorded, first continuously for 30 seconds and then in 10-second strips at 30-second intervals. BP and HR (from ECG) were determined after one and 3 minutes of standing. During the test, the patients were observed for abnormal signs, and after its completion they were queried as to whether AEs had occurred during the test. The symptoms specifically asked about were vertigo, dizziness, fatigue, tremor, sweating, and palpitation.

The changes in systolic ($\Delta SBP_{3–0min}$, mmHg) and diastolic ($\Delta DBP_{3–0min}$, mmHg) BP during the orthostatic test were calculated by subtracting the corresponding supine values

from those measured after 3 minutes of standing. The changes in HR after one (ΔHR_{1-0min}, beats/min) and 3 minutes (ΔHR_{3-0min}, beats/min) of standing were calculated accordingly. The presence or absence of orthostatic hypotension was evaluated according to the recent consensus criteria described above (288). The hemodynamic reaction or joint changes in BP and HR after 3 minutes of standing were also categorized as either normal, vasovagal, hypoadrenergic or hyperadrenergic according to Appenzeller and Oribe (286). After manual measurement of the shortest ($R\text{-}R_{min}$) and the longest ($R\text{-}R_{max}$) R-R intervals from ECG tracings during the first 30 seconds of standing, the corresponding maximum (HR_{max} = 1500 / $R\text{-}R_{min}$) and minimum (HR_{min} = 1500 / $R\text{-}R_{max}$) HRs, and their ratio (HR_{max}/ HR_{min}) were calculated.

Valsalva test

The third test was the Valsalva maneuver performed with the patients seated and connected to an ECG recorder. Each was asked to take a deep breath and then blow into a resistive mouthpiece connected to a mercury manometer, maintaining 40 mmHg (5–6 kPa) airway pressure for 15 seconds (290). A small air leak in the mouthpiece prevented closure of the glottis. A nose clip was also applied. ECG recordings were started 15 seconds before, and continued until 30 seconds after releasing the strain. Corresponding time points were marked on the tracings. Three maneuvres were performed in succession, and the patients rested for 2 minutes between each two attempts. Valsalva ratios were calculated from the longest (phase IV) and the shortest (phase II) R-R intervals that were manually measured from the tracings by the investigator (J.L.) using an ECG ruler. The mean of the three ratios was calculated.

Isometric hand grip test

Before the isometric hand grip test, the maximum voluntary contraction (MVC) of each hand was measured with a calibrated analogue handgrip dynamometer. The hand with the higher MVC (best of three efforts) was selected for the test. Before the test the patients were coached how to maintain the effort, to breath regularly while avoiding the Valsalva maneuver. After a 5-minute rest, ECG and BP were recorded (baseline). The test was performed while seated, and the patients exerted a continuous force of 30% of their MVC on the dynamometer for 3 minutes. A study nurse monitored the dynamometric force for the whole duration of the test. BP and ECG were recorded at one-minute intervals during and one minute after the test. If the patient was unable to complete the 3-minute test, perhaps due to muscle pain or fatigue, BP and ECG were recorded at the time of termination.

The difference in diastolic BP between rest (baseline) and 3 minutes of effort (ΔDBP, mmHg) was calculated.

4.2.4. Bicycle exercise test – cardiorespiratory responses

Course of the exercise test

On each study visit of the third study, a maximal work-conducted exercise test was performed after either overnight withdrawal of L-dopa (control visit, "run-in" test) or 1.5 to 2 hours after study drug intake (second and third study visits). For more details, see the methods in the original publication.

The tests were performed with an electronically braked bicycle ergometer (Bosch) and an incremental workload (steady state) protocol with a 40-W starting load and 40-W increments (Fig. 5). Three-minute stages of constant work rate were chosen in order to achieve steady state for HR and gas-exchange kinetics (291).

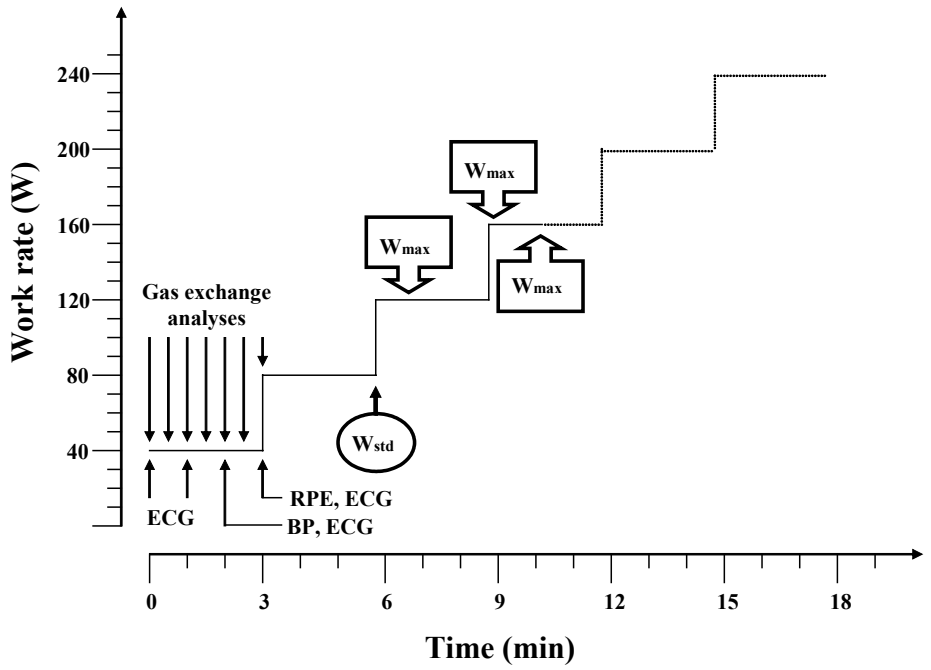


Fig. 5. Protocol of the maximal work-conducted exercise test in the third study. A staged protocol (40 W increments, 3 minutes each) was applied. Repeated assessments of ventilatory gas-exchange parameters, ECG, blood pressure (BP), and rate of perceived exertion (RPE) were performed during each work stage. Each test (three per patient) was continued to subjective maximum (W_{max}), if possible. The submaximal workload standard (W_{std} = the highest completed work stage in all three tests) was determined for each patient individually. One example in Fig. 5: W_{std} = 80W.

Before the test, an indwelling antecubital intravenous catheter was inserted, and blood was sampled (second and third visits) for determination of safety parameters and plasma 3-OMD (Section 4.2.6.). The patients then rested supine for 30 minutes before blood sampling for plasma NA assay (Section 4.2.6.).

During the exercise test (Fig. 5), repeated measurements of BP were done, with continuous recording of ECG and capillary oxygen saturation. Cardiopulmonary auscultation was performed at the end of each work stage. The rate of perceived exertion (RPE) was evaluated at the end of each workload by use of Borg's scale tables (292). With a mask/mouthpiece, gas exchange samples were collected from expired air. The samples were driven through a pneumotachometer and a mixing chamber for continuous extraction into paramagnetic and infrared gas analyzers used, respectively for measurement of O_2 and CO_2 partial pressures. Pneumotachometric spirometry was used in measuring breathing frequency, tidal volume, and minute ventilation (V_E) (EOS Jaeger, Erich Jaeger GmbH,

Würtzburg, Germany). Spiroergometric variables (Table 11) were automatically calculated at 30-second intervals. The test was continued to the subjective maximum (target RPE 19–20/20), unless clinical criteria for discontinuation (293) were met. At peak exercise level, BP and ECG were recorded and blood was sampled for plasma NA assay (Section 4.2.6.).

Table 11. Basic spiroergometric variables of Parkinson's disease patients derived from a maximal bicycle ergometer test.

Variable	*Unit*	*Explanation*
Maximum work load (W_{max}/3')	W	Highest completed work load [W] + (duration at highest achieved work rate [min] / 3 min) × 40 W
Breathing frequency (BF)	1/min	Number of complete breaths per one minute
Tidal volume (V_T)	l, BTPS	Volume of one breath
Minute ventilation (V_E)	l/min, STPD	BF [1/min] × V_T [l, BTPS] × 0,826
F_IO_2, F_EO_2	–	Fractions of O_2 in inspired and expired air, respectively
O_2 uptake (Vo_2)	l/min, STPD	V_E [l/min, STPD] × (F_IO_2 - F_EO_2)[a]
O_2 pulse (Vo_2/HR)	ml/beat, STPD	Vo_2 [l/min, STPD] / HR [beats/min] × 1000 ml/l
F_Eco_2	–	Fraction of CO_2 in expired air
CO_2 output (Vco_2)	l/min, STPD	V_E [l/min, STPD] × F_Eco_2
Respiratory exchange ratio (RER)[b]	–	Vco_2 [l/min, STPD] / Vo_2 [l/min, STPD]
Ventilatory equivalent for O_2	–	V_E [l/min, BTPS] / Vo_2 [l/min, STPD]
Ventilatory equivalent for CO_2	–	V_E [l/min, BTPS] / Vco_2 [l/min, STPD]
Breathing reserve	%	{(MVV [l/min] – V_{Emax} [l/min]) / MVV [l/min]} × 100

W, watt; BTPS, gas volume at body temperature (37 °C) and pressure (47 mmHg) saturated with water vapor; STPD, gas volume at standard temperature (0 °C) and barometric pressure (760 mmHg), dry; O_2, oxygen; HR, heart rate; CO_2, carbon dioxide; MVV, maximal voluntary ventilation at rest; V_{Emax}, minute ventilation at maximum exercise
[a]in dry room air; [b]also known as gas-exchange ratio (R) and respiratory quotient (RQ)

Blood pressure, heart rate, and ECG

Repeated manual measurements of BP (Fig. 5) were done with a brachial cuff, an aneroid manometer (Perfect Aneroid), and stethoscopic auscultation (Korotkoff sounds I and V) from the brachial artery. Systolic and diastolic BP were determined at peak (SBP_{max} and DBP_{max}) and submaximal exercise levels (SBP_{std} and DBP_{std}).

During exercise, ECG recording was done by simulated (Mason-Likar) 12-lead placement (294) with continuous 6-channel ECG monitoring. Paper tracings were produced at 30-sec. intervals. Analyses of HR, arrhythmias, ST-segment deviation (+ 0.06 sec. from J-point), and ST/HR correlation for each lead were done automatically with a computerized ECG device (Case 12, Marquette Inc, U.S.A.). HR was determined at peak exercise (HR_{max}) and at submaximal exercise levels (HR_{std}).

Capillary oxygen saturation (Sao_2)

Sao_2 was monitored from the ear lobe throughout the exercise and the follow-up period with a pulse oximeter (Biox 3700, Ohmeda, U.S.A.). A drop in Sao_2 of ≥3 percentage units from baseline (rest) or below the 90% level was considered clinically significant.

Work capacity

The maximum workload (or power) achieved (W_{max}/3') for an incremental workload protocol was calculated according to Strandell´s formula (Table 11).

Ventilatory gas exchange variables

The between-treatment comparisons of the primary gas exchange variables (Table 11), namely O_2 uptake (Vo_2), O_2 pulse (Vo_2/HR), ventilatory equivalents for O_2 (V_E/Vo_2) and CO_2 (V_E/Vco_2), and the breathing reserve, were carried out at two specific work rates: at peak exercise level, meaning at W_{max}/3', and at a specific submaximal exercise level (W_{std}, see Fig. 5 and legend for details). W_{std} was determined in order to control for the effect of work rate.

Vo_2 is the amount of O_2 extracted from inspired gas in any given period of time. The *maximum O_2 uptake* (Vo_{2peak}) represents the highest Vo_2 achieved during presumed maximal exercise. Vo_{2peak} can be calculated from the gas exchange data as the product of maximum minute ventilation (V_{Epeak}) and the difference between the fractional amounts of O_2 in the inspired (F_IO_2) and expired (F_EO_2) air (Table 11). Vo_2 at submaximal exercise level (Vo_{2std}) is determined accordingly.

O_2 pulse is the amount of O_2 extracted from the blood per stroke volume (SV). It can also be expressed as the product of SV and arteriovenous O_2 difference [$C(a\text{-}v)o_2$]. O_2 pulse is an index of cardiopulmonary capacity:

$$Vo_2/HR\ [ml/beat] = SV \times C(a\text{-}v)o_2 \times 1000\ ml/l$$

O_2 pulse can be calculated by dividing Vo_2 [l/min, STPD] by HR [beats/min]. From the gas exchange data, O_2 pulse at submaximal exercise level (Vo_2/HR_{std}) was determined.

Ventilatory equivalents for O_2 and CO_2 (Table 11) are indices of ventilatory requirement needed in order to extract and eliminate any given amount of O_2 and CO_2. These quotients reflect the appropriateness and efficiency of ventilation at any given metabolic rate. Ventilatory equivalents for O_2 and CO_2 at W_{std} (V_E/Vo_{2std} and V_E/Vco_{2std}) were determined. Breathing reserve is the "unused" fraction of ventilatory reserve at peak exercise and equals the difference (or gap) between maximal voluntary ventilation and V_{Emax}. Breathing reserve (%) was calculated by the formula in Table 11 and the directly measured 15-second maximal voluntary ventilation (Section 4.2.1.).

Respiratory exchange ratio (RER, Table 11) is an indicator of the status of tissue metabolism (aerobic: RER<1, anaerobic: RER>1) at steady state. RER values can therefore serve as rough indices of exercise maximality.

4.2.5. Respiratory muscle strength – maximal airway pressures

In the third study, respiratory muscle strength was assessed during day 2 of the study visits by measurement of maximal static airway pressures (Table 10). While seated, the patients performed three sets of maximal static inspiratory and expiratory efforts through a narrow resistive mouthpiece connected to a pressure sensor. Means (three acceptable efforts) for maximal static inspiratory (P_{Imax}) and expiratory (P_{Emax}) airway pressures were determined from graphic time-pressure plots.

4.2.6. Biochemical determinations

On each study visit (first and third studies), an indwelling catheter was inserted into the antecubital vein for the whole duration of the visit in order to perform repeated blood sampling for biochemical assays. In the second study, a single venous puncture was done for blood sampling.

Plasma catecholamines

During the study visits (first study), venous blood was sampled for determination of plasma concentrations of NA and DA, and of MHPG, a COMT-dependent metabolite of NA. These samples were drawn before (0-value) and 2 hours after study-drug intake (Table 10).

Before the exercise tests (third study) and after a 30-minute rest in a supine position in a sound-controlled room, a 10-ml volume of venous blood was drawn through an indwelling catheter into a vacuum tube for a baseline plasma NA assay (Table 10). The other sample was drawn at peak, or immediately after peak exercise.

In both studies, the blood samples were drawn into tubes containing an Na_2EDTA anticoagulant (Venoject). The sample tubes were kept in an ice bath and centrifuged (3000 rpm for 10 minutes) at +4 °C within 15 minutes of sampling. The separated plasma was pipetted into two plastic tubes which were immediately deep-frozen to -20 °C and stored at -80 °C until delivery for analysis.

All the assays were performed at the Department of Pharmacology, University of Turku. The assays for NA and DA were performed by use of high-pressure liquid chromatography (HPLC) with coulometric electrochemical detection (Coulochem 5100A, ESA inc., Bedford, MA, USA) (295, 296). With the same HPLC apparatus, the MHPG assay was performed according to the method described by Scheinin and co-workers (295) with a slight modification of the method of sample purification and concentration by applying solid-phase extraction with Bond-Elut PH minicolumns (Analytichem International, Harbor City, CA., USA).

The variables assessed were the concentrations of plasma NA, DA, and MHPG at baseline and at 2 hours (first study), and the concentration of plasma NA at rest (C_{NArest}) and at peak exercise (C_{NApeak}), and change in plasma NA (ΔC_{NA}) (third study).

COMT and MAO-B activities

In the first study, venous blood for the determination of S-COMT activity in erythrocytes was sampled on the second and third study visits, first before drug intake (baseline, 0-value) and then one hour after drug intake (Table 10). Each sample consisted of 10 ml of blood collected into an EDTA-tube, which was kept in an ice bath until centrifugation. After centrifugation (1500 g at +4 °C) for 10 minutes, the plasma and the upmost (platelet-rich) cell layer were separated and discarded. The remaining fraction of red blood cells was washed three times by mixing it with ice-cold 0.9% sodium chloride solution of two times the sample volume and centrifuging it at 1500 g and at +4 °C for 10 minutes. The samples were then deep-frozen to the storage temperature of -80 °C until analysis.

Determination of S-COMT activity was done at the Department of Pharmacology, University of Helsinki. Before the assay, the erythrocytes were hemolyzed osmotically on ice after mixing them with cold 1 mM sodium phosphate buffer (pH 7.4) of three times the sample volume. The supernatant was then separated by centrifugation at 20 000 g and at +4 °C for 20 minutes. The assay was performed by HPLC with electrochemical detection using 3,4-dihydroxybenzoic acid as a substrate (297).

Two hours after study drug intake on each study visit, a 10-ml sample of venous blood was drawn into a chilled EDTA tube for determination of platelet MAO-B activity. These tubes were kept in an ice bath and centrifuged at 900 rpm (110 g) and at +5 °C for 30 minutes (Plasma R1000, Jouan, Saint Herblain, France) within one hour of sampling. The platelet-rich fraction (supernatant) was then manually separated with a pipette and transferred to plastic tubes containing 0.1 ml of 100 mM EDTA. These tubes were shaken, and the platelets were precipitated by centrifugation at 3000 rpm (1300 g) and at +5 °C for 15 minutes. After discarding of the supernatant, the platelets in the remaining pellet were dispersed by addition of two ml of cold 0.1 M phosphate buffer (pH 7.4). After re-centrifugation at 2500 rpm (900 g) and at +5 °C for 10 minutes, the supernatant was again discarded, and the walls of the test tubes were dried with cotton swabs. A volume of 0.5 ml of cold 0.1 M phosphate buffer (pH 7.4) was added without mixing into the samples tubes, which were then capped, deep-frozen, and stored at -80 °C until analysis.

The MAO-B activity in the samples was determined radiochemically (1214 Rackbeta, LKB, Wallac, Turku, Finland), with ^{14}C-phenylethylamine as a substrate, at the Bioanalytical laboratory of Orion Pharma, Turku (298, 299). The intra- and inter-assay coefficients of variation (CV) of the method were 4.4% and 11.0%.

Pharmacokinetics of L-dopa and its metabolites

During each study visit of the first study, repeated sampling of blood (Table 10) from the antecubital vein was performed to determine plasma concentration of L-dopa and its metabolites. Each sample consisted of 10 ml blood collected into a chilled vacuum EDTA tube. These tubes were kept in an ice bath and centrifuged at +4 °C. After centrifugation, 500 µl of plasma was pipetted into each of the two Eppendorf tubes containing 25 µl of 10% sodium metabisulfite. After mixing, the tubes were deep-frozen to -20 °C and stored at -80 °C until analysis.

For each time-point of sampling, the plasma concentrations of L-dopa, 3-OMD, DOPAC, and HVA were determined by HPLC with electrochemical detection (300) at the Chemical Research Department of Orion Pharma. The quantization range of the method was 20 to 8000 ng/ml for L-dopa, 200 to 16000 ng/ml for 3-OMD, and 20 to 2000 ng/ml for both DOPAC and HVA. The detection limit of the assay at a signal-to-noise ratio of 3 was 7 pg for L-dopa and DOPAC, 10 pg for 3-OMD, and 6 pg for HVA. The limit of determination was set to 20 ng/ml for all four analytes. The intra-assay CVs for all analytes, i.e., the precision of the method, was <7% at a concentration of 20 ng/ml and <4% at a concentration of 200 and 2000 ng/ml. The inter-assay CV for all analytes was <9% at 80 ng/ml and <6% at 800 ng/ml. The C_{max} and T_{max} of L-dopa were determined directly from the plasma concentration-time data, whereas its $t_{1/2}$ was calculated. The areas under the plasma concentration-time curves for L-dopa, 3-OMD, DOPAC, and HVA from baseline to 6 hours ($AUC_{0–6h}$) were calculated by the linear trapezoidal rule (301).

Pharmacokinetics of entacapone and its Z-isomer

The schedule of blood sampling for determination of entacapone and its Z-isomer from plasma was identical to that of L-dopa and its metabolites (Table 10). The samples were first drawn into vacuum EDTA tubes, then immediately centrifuged at +4 °C, after which 1.5 ml aliquots of plasma were separated and pipetted into glass tubes, deep-frozen to -20 °C, and delivered for analysis at the Chemical Research Department of Orion Pharma. At all stages of the handling and storing process, the samples were shielded against sunlight with thin aluminum foil.

The plasma concentrations of entacapone and its Z-isomer were determined from the samples by similar analytical methods as those for L-dopa and its metabolites (302). The pharmacokinetic parameters (C_{max}, T_{max}, $t_{1/2}$ and AUC_{0-6h}) of entacapone and its Z-isomer were calculated accordingly. The quantization range of the method was 10 to 4000 ng/ml for entacapone and 10 to 500 ng/ml for its Z-isomer. The detection limit of the assay at the signal-to-noise ratio of 3 was 150 pg. The limit of determination was 10 ng/ml for both isomers. The intra-assay CV for both isomers was <10% and ~5% at 10 and 200 ng/ml. For entacapone, the CV was 3% at 4000 ng/ml. The inter-assay CV for entacapone was 15%, <7%, and <3% at 20, 200, and 2000 ng/ml. The inter-assay CV for the Z-isomer was 15% and <3% at 20 and 200 ng/ml.

3-O-methyldopa

Because substantial reduction in 3-OMD formation is observable only after long-term (days or more) treatment with COMT inhibitors (14), plasma 3-OMD can serve as an indicator of treatment effect and compliance. During the last two study visits of the third study, a 5-ml venous blood sample was drawn into an EDTA tube concomitantly with the baseline NA sample. This tube was kept in an ice bath and then centrifuged at +4 °C for 10 minutes no later than 30 minutes after sampling. From the separated plasma, two aliquots of 1.0 ml each were pipetted into Eppendorf tubes containing 25 µl of 20% sodium metabisulfite. The tubes were capped, mixed, deep-frozen to -20 °C, and finally stored at -70 °C until analysis.

The determination of plasma 3-OMD concentration was carried out at the Chemical Research Department of Orion Pharma, Espoo, by reversed-phase HPLC with amperometric detection (300). The limit of quantization of the method was set at 0.10 µg/ml of 3-OMD. The intra-assay CVs at 3-OMD concentrations of 0.10, 0.61, 4.10, and 12.30 µg/ml were 5.8, 2.8, 1.3, and 0.5%.

Blood and urine safety assessments

In addition to assessment of biochemical safety parameters at screening, blood was sampled into EDTA tubes for safety assays after each treatment period, at the end of each washout, and post-study. Assessments included blood count (hemoglobin, leukocytes, erythrocytes, erythrocyte volume proportion, and thrombocytes), serum glucose, sodium, potassium, creatinine, alanine transaminase, aspartate transaminase, gamma glutamyl transferase, alkaline phosphatase, and uric acid. A urine sample was collected for determination of urinary protein and glucose. The biochemical safety parameters were determined according to routine methods at Medix Clinical Laboratories, Kauniainen, Finland. The clinical significance of values outside the corresponding reference range (provided by Medix) was evaluated case-by-case.

4.2.7. Assessment of motor response to L-dopa

In the first two studies, clinical response to treatment was assessed with clinical rating scales. Evaluation of the duration and magnitude of motor response to a drug dose by use of repeated assessments of clinical rating scales constitutes the L-dopa test. Clinical responses were rated in relation to motor disability and dyskinesias. The same observer (J.L.) conducted the scorings throughout the studies.

Unified Parkinson's Disease Rating Scale (UPDRS), Part III

Part III of the UPDRS (Version 3.0) (79), modified slightly by including the grading of arm swings (0 = normal, 1 = possibly diminished, 2 = slightly diminished, 3 = markedly diminished, 4 = absent) (234) to the scale, was used in the assessment of motor response. Each item was scored on a 5-point scale with the total score ranging from zero (normal motor state) to 104 (maximal motor disability). Before drug intake on the mornings of study visits, the scoring was performed either once (second study) or twice at 30-minute intervals (first study, Table 10). This first score (the mean of the two in the first study) constituted the baseline score. After study drug intake, scoring was done at 30-minute intervals over the next 6 hours in both studies. A reduction of >10% in UPDRS motor score from the baseline score was defined as indicating the onset of clinical response (= "starting time"). Likewise, the end of clinical response was considered when the score had returned within <10% of the baseline score (= "end time"). The calculated interval between these two time-points of assessment was defined as the duration of clinical response (= ON-time). The magnitude of clinical response, i.e., the difference between the lowest score of the day and the baseline score, was calculated. The *mean daily motor score*, defined as the mean of the consecutive scores (n=13) over a visit, was calculated (first and second studies). In the second study, the *total daily motor score*, defined as the sum of all consecutive scores (n=13) over visit was also determined.

In the third study, the modified UPDRS motor score was assessed twice during study visits (Table 10), first before and then after exercise. The difference between these two scores was calculated.

Abnormal Involuntary Movement Scale

In the second study, the assessment of clinical response included repeated rating of dyskinesia according to the Abnormal Involuntary Movement Scale (AIMS) (303, 304). AIMS constitutes a rating of dyskinesias in seven different body regions by a 5-point scheme (0 = absent, 1 = possibly present, 2 = mild, 3 = moderate, 4 = severe/disabling). The total AIMS score ranges from 0 (no dyskinesias) to 28 (maximal dyskinesia). The AIMS score was rated in conjunction with assessment of the modified UPDRS motor score (Table 10). The sum of all consecutive scores over a visit was defined as the total daily AIMS score. The highest (peak) score of the day was considered to represent the magnitude of dyskinesias.

4.2.8. Assessment of sleep

In the first two studies, the study visits were preceded by a quantitative assessment of sleep maintenance using 24-hour monitoring of ambulatory motor activity by an accelerometric technique (actigraphy). For that purpose, the patients arrived at the Outpatient Department on each previous morning for the mounting of a wrist-worn activity-monitoring device. The activity monitor (Motionlogger Actigraph® MINI wrist unit, Rev. 1.2, Ambulatory Monitoring, Inc., Ardsley, NY, USA) contained a piezo-electric accelerometer (Vernitron bimorph transducer beam), which quantified mean motor activity over any chosen unit of time (epoch) for storage at a sampling rate of 10 Hz. A Zero Crossing mode (activity count generated with each change in signal voltage in relation to reference voltage) of registration was applied. Epoch length was set to 25 seconds. The wrist unit was always mounted on the same side for each patient, preferably the side with fewer

parkinsonian symptoms. The patients were encouraged to maintain their habitual diurnal pattern of activities, and to remove the unit only when taking a bath. The unit was detached on each study visit morning upon arrival at the Outpatient Department. The device initialization, data retrieval, storage, and analysis were performed with a specific Actigraph Interphase Unit connected to a PC equipped with Action® software (Ver. 1.23, Ambulatory Monitoring Inc.). Sleep actigraphy was supplemented by a patient-rated sleep questionnaire, in which times for "lights-out," awakening, and naps were recorded. From sleep actigraphy and questionnaire data, duration of sleep (from "lights-out" to awakening) and mean motor activity during sleep could be calculated.

4.2.9. Adverse events (AE)

During each study visit, AEs were actively inquired about before drug intake, before discharge (first and second studies), and also before and after each exercise test (third study). They were also inquired about at each safety visit and post-study. AEs were characterized by type, time of onset (and resolution if applicable), temporal course, severity and, if possible, assumed causality regarding ongoing treatment.

The frequency (times reported/observed) of each type of AE, and the number of patients with ≥1 AE per treatment were determined.

4.3. Data management and statistical methods

4.3.1. Data management

The original data were first fed manually into a relational database (Rdb) and then downloaded to Statistical Analysis System (SAS) software in a VAX/VMS environment. The data were listed from the SAS database and crosschecked for errors in data entry by comparison of the lists with the original data before performance of any statistical analyses.

After making corrections to the database, the data were transferred for statistical analysis to either (first and second studies) Consultant Group Covariance Ltd., Helsinki, Finland, using SAS Ver. 6.08 statistical package (SAS Institute Inc., Cary, NC, USA) in a Microsoft Windows environment on a PC, or (third study) Clinical Research Services, Turku, where the analyses were performed with SAS Ver. 6.10.

4.3.2. Statistical methods

First study

Statistical methods were applied in testing for differences in outcome variables between the two study treatments (Table 9), and between each study treatment and control (L-dopa/DDC inhibitor only). The significance level was set at 0.05. Descriptive statistics (mean, standard deviation, median, minimum, and maximum values) and estimates of 95% confidence intervals (CI_{95}) for means were calculated for all discrete and continuous data.

For parametric outcome variables, the analyses were carried out with mixed-model analysis of variance (ANOVA) for crossover design. The normality of distribution was examined graphically using a box-and-whiskers plot and normal probability plot. The goodness-of-fit of covariance matrix was tested by the asymptotic likelihood ratio test. The analyses were performed with the MIXED procedure using type III sums of squares as the basis of inference. Orthogonal contrasts were used in testing for differences between control and the study treatments. Whenever parametric ANOVA was not applicable, e.g., in cases of discrete data or non-normal distribution, a non-parametric signed rank test was applied by use of the NPAR1WAY procedure of SAS. Differences between treatments were then tested with the Kruskal-Wallis test.

Differences between means of hemodynamic (BP, HR) and clinical response variables (mean daily motor score, starting time, ON-time, magnitude of motor response derived from the modified UPDRS part III) were analyzed with mixed-model ANOVA for repeated measures, with the study visit as the repeated factor. ANOVA was used for testing differences in pharmacokinetic outcome variables (except for T_{max}) for L-dopa, its metabolites (3-OMD, DOPAC, and HVA), and entacapone and its Z-isomer, in differences in plasma catecholamine levels (DA, NA, MHPG) and in erythrocyte S-COMT and platelet MAO-B activities. L-dopa T_{max} was tested with the Kruskal-Wallis test, as were differences in duration and mean motor activity during sleep. Descriptive statistics and estimates for CI_{95} of means were calculated for biochemical safety parameters. AEs were characterized by their frequency of occurrence.

Second study

Differences in outcome variables were tested between the three study treatments (Table 9) and between each study treatment and control (L-dopa/DDC inhibitor only). The two-sided significance level (p-value) was set at 0.05. Descriptive statistics were calculated and CI_{95} of means estimated for all discrete and continuous data.

In most cases, a parametric Gaussian mixed-model of ANOVA for a crossover design (305, 306) was applied in the statistical testing for differences between treatments, as well as in testing for period and carry-over effects.

Differences in hemodynamic variables (BP, HR) and in diurnal HR_{mean} (Holter) were tested with the Gaussian mixed-model of ANOVA. Differences in clinical motor (modified motor UPDRS: total daily motor score, starting time, ON-time, magnitude of response) and dyskinesia (total daily AIMS score, peak AIMS score) response variables were tested with ANOVA for repeated measures, followed by the Newman-Keuls test. In the analysis of motor disability, the modified UPDRS motor score at screening served as a covariate. Differences in the duration and mean motor activity during sleep were tested with the Kruskal-Wallis test, and differences in the occurrence of arrhythmias (SVES and VES) during ambulatory ECG by the non-parametric Friedman test. Descriptive statistics and estimates for CI_{95} of means were calculated for biochemical safety parameters. AEs were characterized by their frequency of occurrence, and the differences in number of patients with AEs were tested with McNemar's test.

Third study

When differences in outcome variables between the two study treatments (Table 9), and between each study treatment and control (overnight drug withdrawal) were tested, a p-value <0.05 was considered significant. Outcome variables with normal distribution were summarized by mean, standard deviation, minimum, and maximum, whereas median,

minimum, first quartile, third quartile, and maximum were used in describing variables with non-normal (skewed) distribution.

Baseline comparisons of variables between the two study treatments were carried out with one-way ANOVA (normal distribution) or the Wilcoxon rank sum test (non-normal distribution). For outcome variables with normal distribution, treatment comparisons were accomplished with a model of ANOVA for repeated measures appropriate for the underlying crossover design, incorporating fixed effects for sequence, period, and sequence-by-period interaction. Both the carry-over and the treatment effects were estimated (including CI_{95}) and tested by use of contrasts within this basic model. The statistical comparison between study treatments and control were carried out with the Wilcoxon signed rank test. In case of non-normally distributed variables, the Wilcoxon rank sum test served to detect possible carry-over or period effects.

The comparison of cardiovascular autonomic and cardiorespiratory exercise responses, airway pressures, motor disability, diurnal HR_{mean}, plasma NA, and 3-OMD between study treatments and between study treatments and control were performed with either repeated-measures ANOVA appropriate for cross-over design (normally distributed variables) or the Wilcoxon rank sum and signed rank tests (non-normally distributed variables). In statistical analysis of spiroergometric data, the between-treatment comparisons of each variable were performed between values measured at W_{max}/3' and at W_{std}. Differences in the occurrence of arrhythmias (SVES and VES) during ambulatory ECG were tested with the Wilcoxon signed rank test. Descriptive statistics and estimates for CI_{95} of means were calculated for biochemical safety parameters. AEs were tabulated, and number of patients with AEs was compared between treatments with McNemar's test.

5. RESULTS

5.1. Cardiac rhythm and hemodynamics

5.1.1. Responses during L-dopa test (I, II)

Selegiline had no significant effect on mean daily systolic BP, diastolic BP, or HR in 13 L-dopa-treated PD patients on entacapone after repeated dosing of the drugs for 2 weeks (I). In comparison to control, the mean daily values for systolic and diastolic BP (both supine and standing) were reduced ($p < 0.01$–0.001) after 2 weeks of entacapone therapy, either with or without selegiline, whereas mean daily HR remained unchanged (Table 3 in publication I, as "Table 3/I").

No statistically significant differences appeared in mean daily systolic BP, diastolic BP, or HR (Table 12), or in their mean daily changes (systolic BP and HR) during the orthostatic test (Table 1/II) between selegiline, entacapone, or entacapone + selegiline after 2-week administration of these treatments as adjuncts to L-dopa in 16 PD patients (II).

No clinically significant ECG changes were observable (I, II). The abnormal findings (one of each) already present at screening, i.e., first degree atrioventricular block (I), left anterior fascicular block (I), right bundle-branch block (II), and left ventricular hypertrophy (II), remained unchanged throughout the studies.

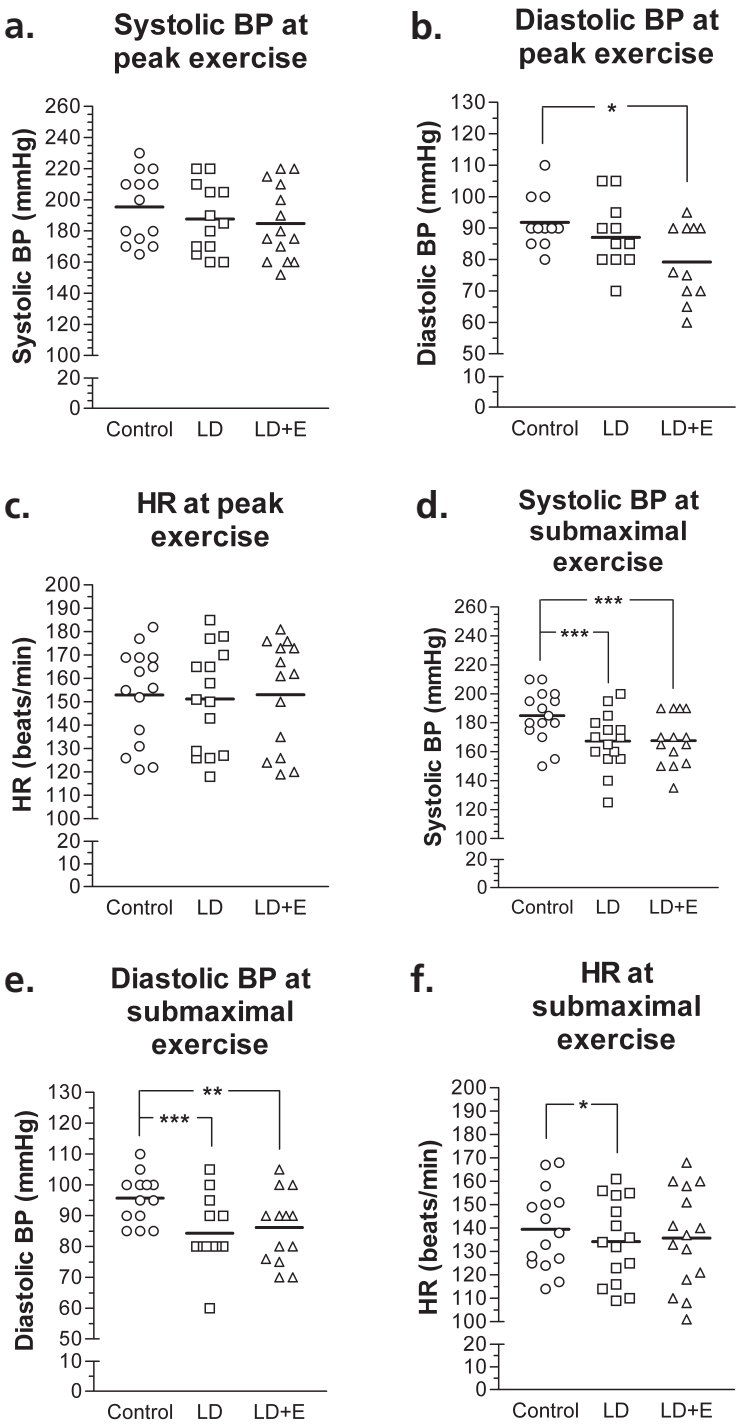
Table 12. Daily values (mean ± SD) for systolic blood pressure (BP), diastolic BP, and heart rate (HR) during orthostatic testing (II) of Parkinson's disease patients.

Daily BP and HR	*Treatment*			
	LD (control)	LD + E	LD + S	LD + E + S
Systolic BP, supine (mmHg)	142 ± 13	134 ± 12	133 ± 8	136 ± 11
Systolic BP, standing (mmHg)	128 ± 19	120 ± 17	118 ± 16	120 ± 12
Diastolic BP, supine (mmHg)	82 ± 8	77 ± 7	77 ± 5	78 ± 7
Diastolic BP, standing (mmHg)	77 ± 13	72 ± 11	72 ± 10	72 ± 11
HR, supine (beats/min)	68 ± 9	64 ± 9	65 ± 10	64 ± 10
HR, standing (beats/min)	76 ± 11	72 ± 9	75 ± 8	74 ± 9

Assessments were done first before (control), and then after 2 weeks on each study treatment. LD, L-dopa; E, entacapone; S, selegiline. N=14 for each group. Differences between study treatments not statistically significant.

5.1.2. Responses to maximal exercise (IV)

For 15 PD patients, peak exercise values for systolic BP, diastolic BP, or HR were not significantly changed after one-week treatment with entacapone when compared to one-week treatment with placebo, both given as adjuncts to L-dopa (Figs. 6a-c). Lower values for diastolic BP at peak exercise ($p < 0.05$) were measured after entacapone with L-dopa when compared to those measured during the control (run-in) test after overnight withdrawal of L-dopa (Fig. 6b). The differences in either systolic BP or HR at peak exercise were not statistically significant between control and study treatments (Figs. 6a and 6c).



Figs. 6a-f. Group means (heavy horizontal bars) and individual values (symbols) for systolic blood pressure (BP), diastolic BP and heart rate (HR) measured both at peak (Figs. 6a-c) and at submaximal (Figs. 6d-f) exercise level (IV), first after overnight withdrawal of L-dopa (Control, O), and then after one week on either entacapone 200 mg t.i.d./q.i.d. (LD+E, Δ) or placebo (LD, □) as adjuncts to each dose of L-dopa. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

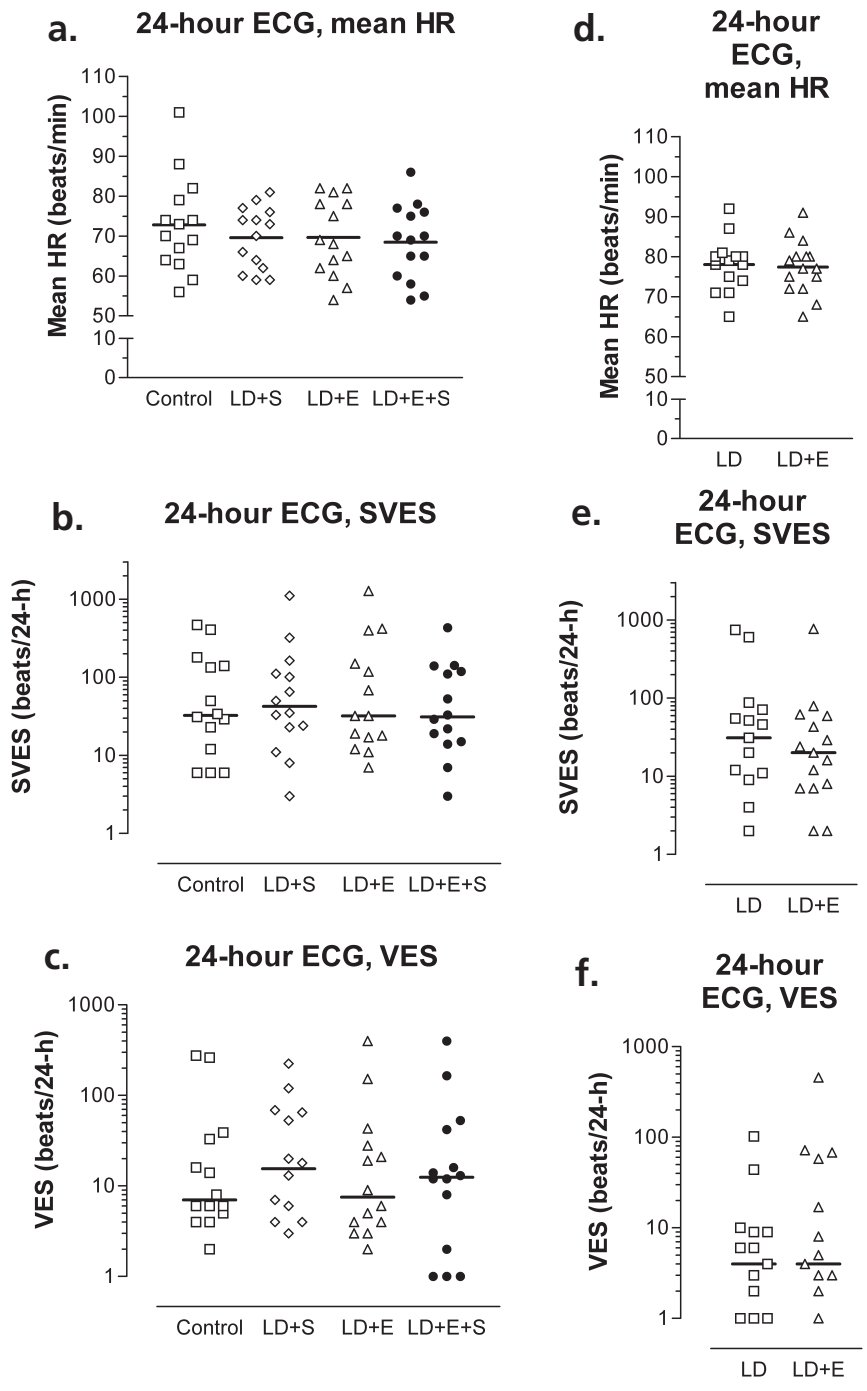
Entacapone, when compared to placebo, showed no significant effect on systolic BP, diastolic BP, or HR at a submaximal exercise level (Figs. 6d-f). Systolic and diastolic BPs at submaximal exercise level were lower ($p < 0.01$ - 0.001) after L-dopa, both with and without entacapone (Figs. 6d and 6e, respectively). HR at submaximal exercise level was lower ($p < 0.05$) after placebo + L-dopa, whereas the difference between control and entacapone + L-dopa was not statistically significant (Fig. 6f).

Co-administration of entacapone with L-dopa was not associated with clinically significant changes in exercise ECG. None of the findings observed during the tests were symptomatic or required intervention (e.g., medication, premature termination of exercise, extended follow-up). No clinically significant cardiac conduction abnormalities or arrhythmias occurred. Two patients had monofocal ventricular ectopic beats at peak exercise: one after entacapone + L-dopa, and the other both during control and after placebo + L-dopa. Each of the exercise tests in one study patient (62-year-old male) was associated with asymptomatic, low-amplitude, work-rate-dependent horizontal ST-segment depression. He had neither a history of ischemic heart disease nor pre-disposing risk factors for cardiovascular disease; the findings, consistent with myocardial ischemia, demonstrated rapid normalization during post-exercise follow-up.

5.1.3. Ambulatory ECG (II, IV)

Administration of entacapone, selegiline, or both together as adjuncts to L-dopa for 2 weeks (II) did not significantly change the mean ambulatory HR of 14 patients with PD (Fig. 7a). No significant differences in the occurrence of supraventricular (Fig. 7b) or ventricular (Fig. 7c) extrasystoles emerged, either between the study treatments or between study treatments and control. Occurrence of non-sustained ventricular tachycardia (Table 2/II) was not increased with entacapone therapy, either with or without selegiline.

When compared to placebo + L-dopa, one-week administration of entacapone with each dose of L-dopa (IV, unpublished results) changed neither mean HR (Fig. 7d) nor the occurrence of supraventricular (Fig. 7e) nor ventricular (Fig. 7f) extrasystoles.



Figs. 7a-f. Group means (bars in Figs. 7a,d), medians (Figs. 7b-c,e-f), and individual values (symbols) for mean diurnal heart rate (HR) and the number of supraventricular (SVES) and ventricular (VES) extrasystoles during a 24-hour ambulatory ECG registration. Second study (Figs. 7a-c): first during L-dopa (Control,□), and then after 2 weeks of either selegiline 10 mg o.d. (LD+S, ◊), entacapone 200 mg t.i.d/q.i.d. (LD+E, Δ), or entacapone plus selegiline (LD+E+S, ●) as adjuncts to L-dopa. Third study (Figs. 7d-f): after one week of either entacapone 200 mg (LD+E, Δ) or placebo (LD, □), both given t.i.d./q.i.d. as adjuncts to each dose of L-dopa. No significant differences between groups. Log scale Y-axis in Figs 7b-c,e-f.

5.2. Effects on cardiovascular autonomics (III)

5.2.1. Responses to deep breathing

Mean HR variation during deep voluntary breathing was not significantly altered by one-week therapy with entacapone as an adjunct to each dose of L-dopa when compared to the corresponding placebo regimen (Table 13). Differences between study treatments and control were also non-significant.

Based on the categorization of individual HR responses to deep breathing by Piha (287), the number of non-normal (borderline/pathologic) responses was similar between control (2/1), placebo plus L-dopa (2/1), and entacapone plus L-dopa (2/0). The remaining majority of HR responses were considered normal.

Table 13. Parameters (mean ± SD) of cardiovascular autonomic function (III).

Function tests of the cardiovascular autonomic nervous system	*Treatment*		
	Control	LD + P	LD + E
Deep breathing test			
• Deep breathing difference (beats/min)	11.9 ± 6.5	12.3 ± 8.3	12.7 ± 7.0
Orthostatic test			
• HR_{max}/HR_{min}	[1]1.18 ± 0.06	[2]1.18 ± 0.15	[1]1.16 ± 0.16
• $\Delta SBP_{3\text{-}0min}$ (mmHg)	-5.5 ± 12.0	-8.5 ± 17.1	-14.5 ± 28.3
• $\Delta DBP_{3\text{-}0min}$ (mmHg)	5.0 ± 6.2	[2]3.4 ± 7.8	13.7 ± 11.2
• $\Delta HR_{3\text{-}0min}$ (beats/min)	14.5 ± 8.3	15.5 ± 9.2	15.1 ± 11.4
• $\Delta HR_{1\text{-}0min}$ (beats/min)	13.6 ± 6.9	15.9 ± 8.0*	16.2 ± 10.8
Valsalva test			
• Valsalva ratio	1.34 ± 0.24	1.34 ± 0.30	1.39 ± 0.30
Isometric hand grip test			
• ΔDBP (mmHg)	15.5 ± 9.6	14.9 ± 8.9	13.0 ± 8.1

Assessments were done first after overnight withdrawal of L-dopa (control), and then after one week on L-dopa with either entacapone 200 mg or placebo. LD, L-dopa; P, placebo; E, entacapone; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR_{max}/HR_{min}, maximum orthostatic R-R interval variation; Δ, change (over time period).
[1]N=13, [2]N=14, otherwise N=15. Control vs. placebo plus L-dopa: *, $p < 0.05$.

5.2.2. Responses to orthostatic challenge

Entacapone had no effect on the maximum orthostatic R-R interval (or HR) variation when compared to placebo, when both were given as adjuncts to L-dopa for one week (Table 13). In comparison to maximum HR variation measured during the control test, L-dopa did not affect this ratio either with or without entacapone.

The number of borderline abnormal/pathologic responses in maximum HR variation during the orthostatic test (287) was 2/0 during control, 5/1 after entacapone plus L-dopa, and 4/1 after placebo plus L-dopa. All the other responses were classified as normal.

Differences in the BP and HR parameters were not statistically significant between entacapone and placebo, or between study treatments and control, with the exception of the HR change during one minute being higher ($p < 0.05$) after placebo plus L-dopa than during control treatment (Table 13).

One patient had systolic orthostatic hypotension during the control test, whereas this was observed in three patients after administration of entacapone and four after placebo, in conjunction with L-dopa (Table 2/III). Diastolic orthostatic hypotension was apparent in two patients after entacapone plus L-dopa and in one after placebo plus L-dopa, but not during the control test.

L-dopa-related symptomatic orthostatic hypotension, either systolic or diastolic, appeared in two patients, neither of which had orthostatic hypotension during the control test. One of these developed symptomatic orthostatism with dizziness after both entacapone (55 and 10 mmHg reductions in systolic and diastolic BP) and placebo (42 and 13 mmHg reductions). The other patient had symptomatic orthostatism (26 mmHg systolic BP decrease) after L-dopa with placebo, but not after L-dopa with entacapone.

One patient demonstrated systolic orthostatic hypotension after entacapone plus L-dopa that was not observable after administration of placebo plus L-dopa. This event was asymptomatic. In another patient, a consistent and marked systolic orthostatism occurred during each test, including control (–40 mmHg), after entacapone plus L-dopa (–100 mmHg), and after placebo plus L-dopa (–42 mmHg). She was, however, symptomatic only during the control test.

Normal hemodynamic reactions after 3 minutes of standing were observable in 13 patients during control, in 11 patients after L-dopa with entacapone, and in 10 patients after L-dopa with placebo. A hypoadrenergic reaction occurred in one patient during control and in three and four patients after L-dopa with either entacapone or placebo, respectively. A hyperadrenergic reaction was consistently evident in one patient, both during control and after each study treatment.

5.2.3. Responses to the Valsalva maneuver

No significant differences in the Valsalva ratio determined after L-dopa intake were observable between entacapone and placebo, when these were administered with each dose of L-dopa for one week. Neither were there significant differences between control and the study treatments (Table 13).

In comparing these results with reference values for the Valsalva ratio (287), a borderline abnormal response was evident in five patients during control, in two after entacapone plus L-dopa, and in five after placebo plus L-dopa. One pathologic response occurred after each study treatment; other responses were considered normal.

5.2.4. Responses to sustained isometric effort

No differences appeared in diastolic BP change during 3 minutes of sustained isometric effort between entacapone and placebo, after administration of each in conjunction with L-dopa for a one-week period, nor between the control and either of these study treatments (Table 13).

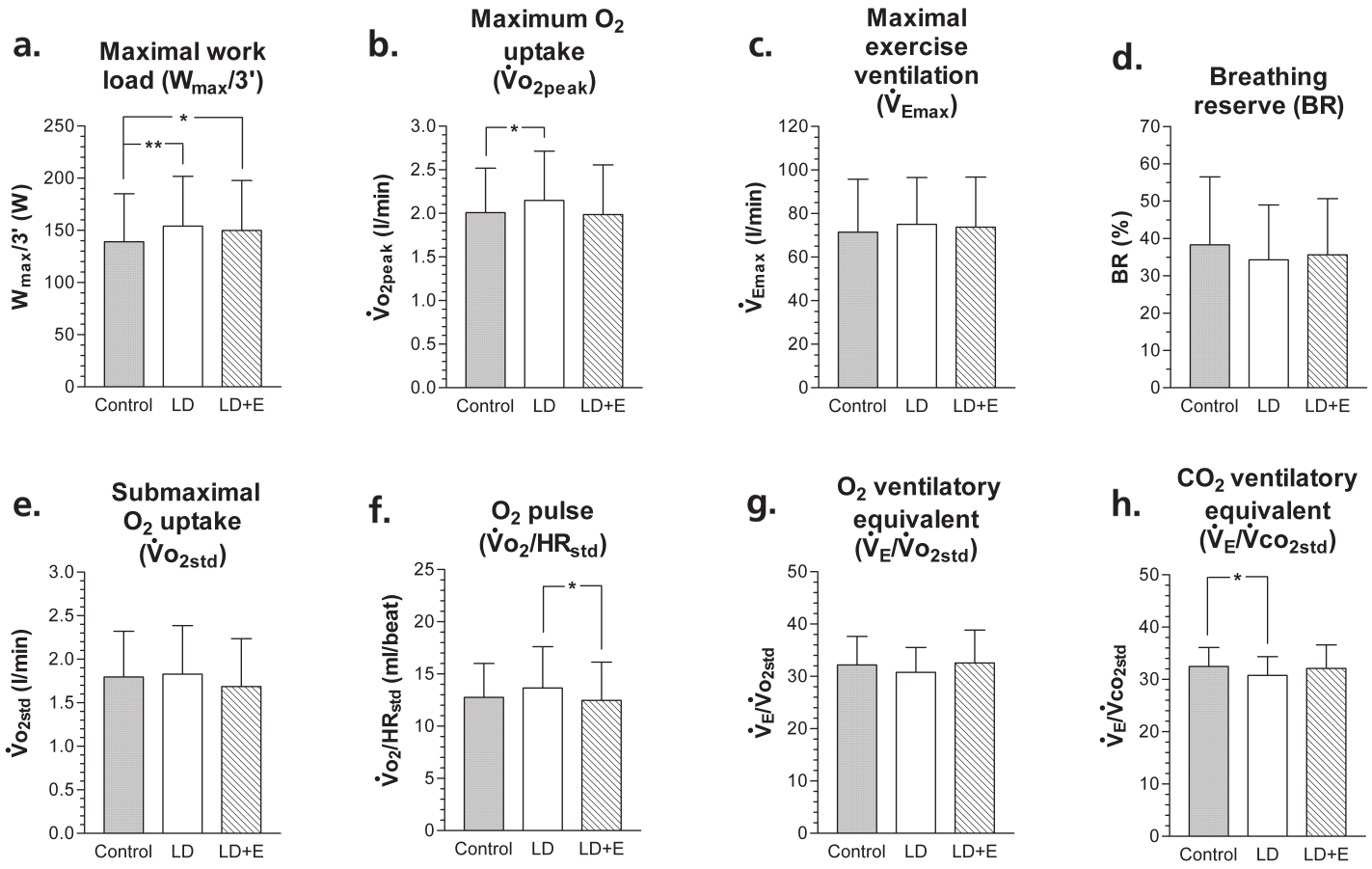
Comparison of results of diastolic BP change with reference values for isometric effort (287) showed borderline abnormal responses in five patients during the control, in four after entacapone plus L-dopa, and in five after placebo plus L-dopa. A pathologic response occurred in two patients after entacapone plus L-dopa, and in another two after placebo plus L-dopa. Other values fell within the range for the predicted normal response.

5.3. The effects on cardiorespiratory performance (IV)

5.3.1. Gas exchange responses to maximal exercise

The maximum O_2 uptake was not changed by adding entacapone to L-dopa treatment (Fig. 8b). Maximum O_2 uptake was higher ($p < 0.05$) after placebo plus L-dopa than during control. The difference between entacapone plus L-dopa and control was not significant.

The differences in either maximal exercise ventilation (Fig. 8c) or breathing reserve (Fig. 8d) were not statistically significant between the two study treatments, or between both study treatments and control.



Figs. 8a-h. Variables (mean + SD) of gas exchange and cardiorespiratory capacity assessed at both peak (Figs. 8a-d) and submaximal (Figs. 8e-h) exercise level (IV), first after overnight withdrawal of L-dopa, (Control), and then after one week of either entacapone 200 mg (LD+E) or placebo (LD), both given t.i.d./q.i.d. as L-dopa adjuncts. Std, submaximal (standardized) workload (See Fig. 5). N=14-15 for each group. *, $p < 0.05$; **, $p < 0.01$.

5.3.2. Gas exchange responses to submaximal exercise

Entacapone had no significant effect on the submaximal, work-rate standardized O_2 uptake measured 1.5 to 2 hours from L-dopa intake in this sample of 15 patients with PD, nor were there any differences in the submaximal O_2 uptake between control and the study treatments (Fig. 8e). Lower values ($p < 0.05$) for submaximal O_2 pulse were observable after entacapone plus L-dopa than after placebo plus L-dopa. Differences in submaximal O_2 pulse were not significant between control and either study treatment (Fig. 8f).

After administration of the study drugs with L-dopa, entacapone did not significantly change the ventilatory equivalents for either O_2 or CO_2 at submaximal exercise level when compared to placebo (Figs. 8g and 8h, respectively). Submaximal ventilatory equivalent for CO_2 was lower than control values ($p < 0.05$) after placebo plus L-dopa, but not after entacapone plus L-dopa.

5.3.3. The effects on work capacity

When compared to placebo, entacapone did not change the maximal workload at 1.5 to 2 hours from its intake with L-dopa in this sample of PD patients (Fig. 8a). Higher values for maximal workload were achieved after L-dopa intake, both with entacapone ($p < 0.05$) and with placebo ($p < 0.01$), than during the control test without L-dopa.

5.3.4. Maximal airway pressures

No significant differences appeared in the means for maximal inspiratory airway pressure between entacapone (12.9 ± 3.0 kPa) and placebo (12.7 ± 3.5 kPa), each administered as an L-dopa adjunct for one week, or between treatments and control (12.0 ± 2.6 kPa).

The mean for maximal expiratory airway pressure was not significantly changed after one-week therapy with entacapone plus L-dopa (20.8 ± 4.5 kPa) when compared to placebo plus L-dopa (19.1 ± 4.8 kPa). Neither of these differed significantly from control values (19.3 ± 4.6 kPa).

5.4. The effects on plasma catecholamines

5.4.1. Catecholamines and metabolites during L-dopa test (I)

Regardless of the use of selegiline, the 2-week co-administration of entacapone with L-dopa in 13 PD patients did not alter the plasma concentration of NA or DA measured both before and 2 hours after intake of the study drugs (Table 14). Two hours after administration of entacapone with L-dopa – both with and without selegiline – a significant decrease ($p < 0.01 – 0.001$) in the concentration of a COMT-dependent NA metabolite, MHPG, was, however, observable (Table 14).

Table 14. Plasma concentrations (mean ± SD) of noradrenaline (NA), dopamine (DA), and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) (I).

Plasma catecholamines	*Treatment*		
	LD (Control)	LD + E	LD + E + S
NA (nmol/l)			
• 0 h	2.62 ± 1.32	2.02 ± 0.94	1.93 ± 0.96
• 2 h	1.25 ± 0.57	1.01 ± 0.50	1.16 ± 0.67
DA (nmol/l)			
• 0 h	1.02 ± 0.82	1.29 ± 0.46	1.95 ± 1.21
• 2 h	3.57 ± 2.44	4.30 ± 3.42	4.96 ± 2.81
MHPG (nmol/l)			
• 0 h	17.75 ± 6.25	14.42 ± 4.21	12.75 ± 3.11
• 2 h	16.42 ± 4.89	11.58 ± 3.53**	[1]10.27 ± 2.28***

Assessments were done first with L-dopa (control), and then after 2 weeks on L-dopa + entacapone either with or without selegiline. LD, L-dopa; E, entacapone; S, selegiline. [1]N=11, otherwise N=12. Timing of assessment: before (0h), and 2 hours after (2h) drug intake. Control vs. study treatments: **, $p < 0.01$; ***, $p < 0.001$.

5.4.2. Plasma NA response to exercise (IV)

One-week therapy with entacapone as an adjunct to L-dopa had no significant effect on either recumbent or peak exercise levels of plasma NA in 15 PD patients (Table 15), nor were there significant differences between values measured during the run-in test (without L-dopa) and those measured after L-dopa administration, regardless of the use of entacapone. In comparison to recumbent levels of plasma NA, approximately ten-fold increases in its concentration occurred at peak exercise, both during control and after both study treatments (Table 15).

Table 15. Rest and peak exercise levels (mean ± SD) of plasma noradrenaline (NA) during a maximal work-conducted exercise test (IV).

Plasma noradrenaline	*Treatment*		
	Control	LD + P	LD + E
NA (nmol/l)			
• Rest	1.87 ± 0.83	1.72 ± 0.73	1.79 ± 0.78
• Peak exercise	18.67 ± 12.65	21.33 ± 15.69	21.46 ± 15.65

Assessments were done first after withholding L-dopa overnight (control), and then after one week on L-dopa with either entacapone 200 mg or placebo. LD, L-dopa; P, placebo; E, entacapone. N=15 for each group. No significant differences between treatments.

5.5. Pharmacokinetic and dynamic responses

5.5.1. Inhibition of S-COMT and MAO-B activities (I)

Entacapone, one hour from its administration with L-dopa, significantly reduced ($p < 0.001$) the activity of S-COMT in red blood cells (Fig. 9). The mean decrease in activity from control was 38% after entacapone plus placebo and 36% after entacapone plus selegiline. Selegiline had no effect on S-COMT activity, either at baseline or after one hour from intake.

After administration of selegiline for 2 weeks, practically total (> 99.9%) inhibition of platelet MAO-B activity was observable ($p < 0.001$). Entacapone had no effect on platelet MAO-B activity (Fig. 10).

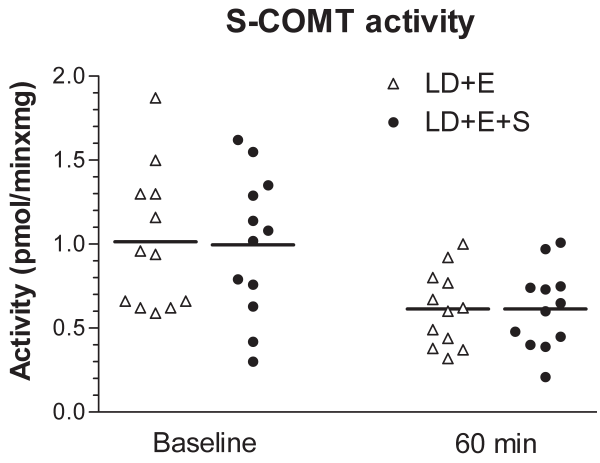


Fig. 9. Group means (bars) and individual values (symbols) for soluble catechol-*O*-methyltransferase (S-COMT) activity in red blood cells (I), determined before (baseline) and 60 minutes after intake of morning doses of entacapone 200 mg plus L-dopa, with either selegiline 10 mg (LD+E+S) or placebo (LD+E). Baseline vs. 60 min.: $p < 0.001$. Differences between selegiline and placebo not significant.

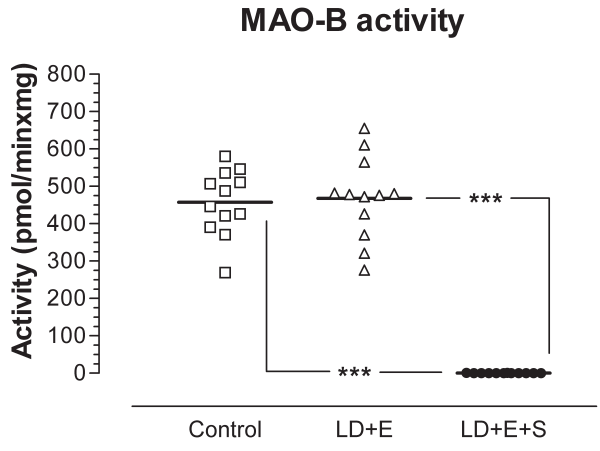


Fig. 10. Group means (bars) and individual values (symbols) for platelet monoamine oxidase type B (MAO-B) activity (I) at 2 hours from study drug intake, first after L-dopa (Control, □), and then after 2 weeks of L-dopa with either selegiline 10 mg o.d. plus entacapone 200 mg t.i.d./q.i.d. (LD+E+S, ●), or placebo o.d. plus entacapone 200 mg t.i.d./q.i.d. (LD+E, Δ). ***, $p < 0.001$.

5.5.2. Pharmacokinetics of L-dopa and its metabolites (I)

The bioavailability of L-dopa was significantly enhanced ($p < 0.001$) by entacapone 200 mg, both with and without selegiline (Table 16, Fig. 3a/I). Entacapone had no significant effect on either the C_{max} or T_{max} of L-dopa (Table 16). In comparison to control, the plasma $t_{1/2}$ of L-dopa was slightly higher after administration of entacapone, either with or without selegiline, as an adjunct to L-dopa. This increase was, however, significant only after entacapone plus selegiline ($p < 0.05$). Selegiline had no significant effect on L-dopa pharmacokinetics in entacapone-treated PD patients.

After 2 weeks of treatment with entacapone, significant decreases ($p < 0.001$) occurred in the bioavailability of both 3-OMD and HVA, the COMT-dependent metabolites of L-dopa and DA (Table 16, Figs. 3b,d/I). Selegiline had no effect on the bioavailability of either of these metabolites in entacapone-treated PD patients.

Table 16. Pharmacokinetic parameters (mean ± SD) for L-dopa and its metabolites 3-O-methyldopa (3-OMD), dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA) (I).

Pharmacokinetics: L-dopa & metabolites	*Treatment*		
	LD (control)	LD + E	LD + E + S
L-dopa			
• $AUC_{0\text{-}6h}$ (ng/ml x h)	5982 ± 1413	9106 ± 2653***	8604 ± 2071***
• $t_{1/2}$ (h)	1.2 ± 0.2	1.3 ± 0.2	1.4 ± 0.3*
• T_{max} (h)	1.3 ± 1.0	1.5 ± 1.0	1.5 ± 1.1
• C_{max} (ng/ml)	3201 ± 1472	3424 ± 1196	3118 ± 779
3-OMD			
• $AUC_{0\text{-}6h}$ (ng/ml x h)	47210 ± 16434	27448 ± 12537***	23248 ± 9503***
DOPAC			
• $AUC_{0\text{-}6h}$ (ng/ml x h)	98 ± 61	264 ± 88***	†††185 ± 85***
HVA			
• $AUC_{0\text{-}6h}$ (ng/ml x h)	537 ± 149	476 ± 171***	406 ± 142***

Assessments were done first after L-dopa (control), and then after 2 weeks on L-dopa plus entacapone 200 mg t.i.d./q.i.d. plus selegiline 10 mg/placebo o.d. LD, L-dopa; E, entacapone; S, selegiline; $AUC_{0\text{-}6h}$, area under the plasma concentration-time curve over 6 hours from drug intake; $t_{1/2}$, plasma elimination half-life; T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration. N=12 for each group.
Control vs. study treatments: *, $p < 0.05$; ***, $p < 0.001$. Selegiline vs. Placebo: †††, $p < 0.001$.

The bioavailability of DOPAC, the MAO-dependent metabolite of DA, was significantly increased ($p < 0.001$) after addition of entacapone, either with or without selegiline, to L-dopa therapy. This increase was significantly less pronounced after selegiline ($p < 0.001$) than after placebo (Table 16, Fig. 3c/I).

5.5.3. Pharmacokinetics of entacapone and its Z-isomer (I)

In 12 L-dopa-treated PD patients, the repeated dosing of the MAO-B inhibitor selegiline for 2 weeks had no effect on the main pharmacokinetic parameters of either entacapone or the Z-isomer of entacapone (Table 17).

Table 17. Pharmacokinetic parameters (mean ± SD) of entacapone and its Z-isomer (I).

Pharmacokinetics: entacapone & its Z-isomer	*Treatment*	
	LD + E	LD + E + S
Entacapone		
• $AUC_{0\text{-last}}$ (ng/ml x h)	1517 ± 447	1399 ± 553
• T_{max} (h)	0.8 ± 0.7	1.0 ± 0.8
• C_{max} (ng/ml)	1232 ± 503	1079 ± 600
Z-isomer		
• $AUC_{0\text{-last}}$ (ng/ml x h)	102 ± 36	104 ± 51
• T_{max} (h)	0.8 ± 0.7	1.2 ± 1.0
• C_{max} (ng/ml)	76 ± 35	74 ± 43

Assessments were done after 2 weeks on L-dopa plus entacapone 200 mg t.i.d./q.i.d. plus either selegiline 10 mg or placebo o.d. LD, L-dopa; E, entacapone; S, selegiline; $AUC_{0\text{-}6h}$, area under the plasma concentration-time curve over 6 hours, T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration. N=12 for each group. No significant differences between treatments.

5.5.4. Plasma concentration of 3-OMD (IV)

When administered as L-dopa adjuncts for one-week, the concentration of plasma 3-OMD (mean ± SD), the COMT-dependent metabolite of L-dopa, was significantly lower ($p < 0.001$) after entacapone (1.24 ± 0.55 µg/ml) than after placebo (2.58 ± 1.08 µg/ml).

5.6. Effects on clinical response to L-dopa

5.6.1. Motor response to the L-dopa test (I, II)

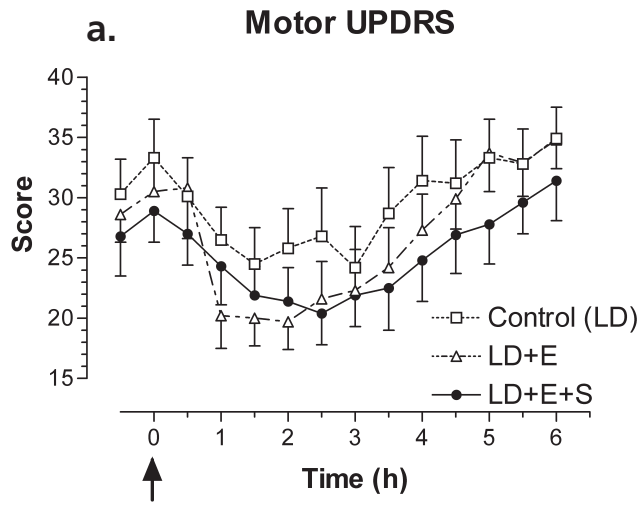
According to repeated assessments of UPDRS part III, the mean daily motor score of 13 PD patients (I) was significantly lower ($p < 0.01$) after concomitant administration of entacapone with selegiline than without selegiline as adjuncts to L-dopa (Table 18, Fig. 11a). The mean daily motor score was also significantly lower ($p < 0.001$) after both study treatments than after control therapy with L-dopa only. No differences appeared in time of onset, duration, or magnitude of motor response between the study treatments or between treatments and control (Table 18).

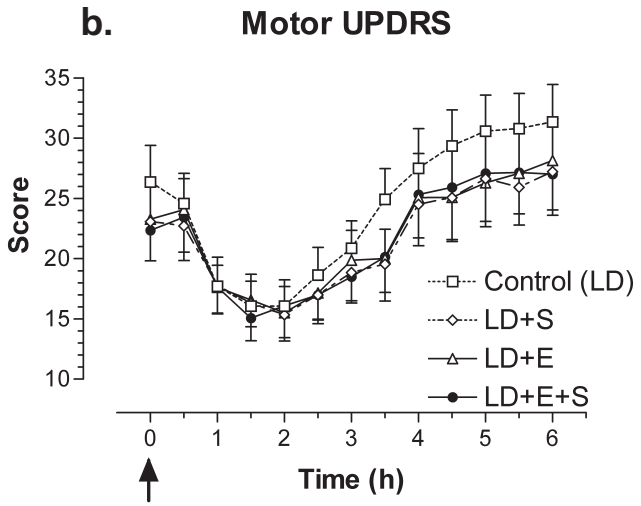
Table 18. Parameters (mean ± SD) of clinical response to L-dopa based on the motor part of the Unified Parkinson's Disease Rating Scale (UPDRS) (I).

Clinical response: motor UPDRS	*Treatment*		
	LD (control)	LD + E	LD + E + S
Mean daily score[a]	29.6 ± 11.2 (165)	26.9 ± 10.4*** (168)	[††]25.4 ± 10.4*** (168)
Magnitude of response[b]	-10.4 ± 6.1 (12)	-13.1 ± 5.0 (12)	-10.5 ± 4.3 (12)
Starting time[c] (h)	1.0 ± 0.6 (11)	1.1 ± 0.7 (11)	1.4 ± 0.8 (12)
ON-time[d] (h)	3.1 ± 1.4 (11)	2.9 ± 0.8 (11)	3.0 ± 1.3 (12)

Ratings were done repeatedly at half-hour intervals over 6 hours from drug intake, first after L-dopa (control), and then after 2 weeks on L-dopa plus entacapone 200 mg t.i.d./q.i.d. plus selegiline 10 mg/placebo o.d. LD, L-dopa; E, entacapone; S, selegiline. [a]Arithmetic mean of the 13 motor scores over study visit; [b]lowest (best) motor score of the day minus the baseline score; [c]onset of clinical response, i.e., time from drug intake when a reduction (improvement) in motor score of >10% from baseline score was observed; [d]duration of clinical response = end time (time from drug intake when the difference between motor score and the baseline score ≤10% of the baseline score) – starting time. Figures in parentheses indicate number of assessments in each group.
Control vs. study treatments: ***, $p < 0.001$. Selegiline vs. Placebo: [††], $p < 0.01$.

No significant differences in mean daily motor score (Table 19) emerged between entacapone, selegiline, or entacapone plus selegiline in 14 PD patients (II). Differences in mean daily motor score between control and each study treatment were also non-significant. In comparison to control, the total daily motor score ("AUC" of motor disability) was significantly reduced ($p < 0.05$ for all contrasts) after entacapone (by 10%), selegiline (by 12%) and entacapone plus selegiline (by 11%) as add-on therapies to L-dopa (Table 19, Fig. 11b). Differences in the total daily motor score between study treatments were non-significant. Neither time of onset, duration, nor magnitude of motor response was significantly changed by any of the study treatments (Table 19).





Figs. 11a-b. Modified motor (part III) subscores (mean ± SEM) of the Unified Parkinson's Disease Rating Scale (UPDRS), assessed first before (0 h), and then repeatedly after intake of drugs (arrow) in Studies 1 (Fig. 11a, n=10-12) and 2 (Fig. 11b, n=13-14). The first assessment was done after intake of L-dopa (Control), and then after 2 weeks of L-dopa with entacapone 200 mg t.i.d./q.i.d. (LD+E), entacapone 200 mg t.i.d./q.i.d. plus selegiline 10 mg o.d. (LD+E+S), or selegiline 10 mg o.d. (LD+S, in Study 2). Higher score indicates greater motor disability. See Section 5.6.1. and Tables 18 and 19 for analyses of results.

Table 19. Parameters (mean ± SD) of clinical response to L-dopa based on the motor part of the Unified Parkinson's Disease Rating Scale (UPDRS) and the Abnormal Involuntary Movement Scale (AIMS) (II).

Clinical response: motor UPDRS & AIMS	*Treatment*			
	LD (control)	LD + E	LD + S	LD + E + S
Motor UPDRS				
• Mean daily score[a]	24.2 ± 10.9 (182)	22.0 ± 11.2 (180)	21.6 ± 11.3 (180)	21.8 ± 10.6 (180)
• Total daily score[b]	315 ± 112 (182)	283 ± 120* (180)	277 ± 127* (180)	280 ± 116* (180)
• Magnitude[c]	-11.6 ± 8.3 (14)	-9.6 ± 6.4 (14)	-10.1 ± 7.4 (14)	-8.4 ± 6.3 (14)
• Starting time[d] (h)	1.1 ± 0.3 (14)	1.3 ± 0.5 (14)	1.4 ± 0.9 (14)	1.1 ± 0.2 (12)
• ON-time[e] (h)	2.6 ± 0.8 (10)	2.2 ± 1.4 (10)	2.0 ± 1.2 (10)	2.3 ± 0.7 (10)
AIMS				
• Total daily score[f]	8.6 ± 12.6 (11)	13.8 ± 15.9 (11)	12.6 ± 17.7 (11)	16.8 ± 19.5**(11)
• Peak score[g]	2.4 ± 2.7 (11)	3.4 ± 3.6 (11)	3.5 ± 3.6 (11)	3.9 ± 3.4 (11)

Ratings were done repeatedly at half-hour intervals over 6 hours from drug intake, first after L-dopa (control), and then after 2 weeks on L-dopa plus entacapone 200 mg t.i.d./q.i.d., L-dopa plus selegiline 10 mg o.d., or L-dopa plus entacapone plus selegiline. LD, L-dopa; E, entacapone; S, selegiline; [a]arithmetic mean of the 13 motor scores over study visit; [b]sum of all the 13 motor scores over study visit; [c]lowest (best) motor score of the visit minus the baseline score; [d]onset of clinical response, i.e., time from drug intake when a reduction (improvement) in motor score of >10% from baseline score was observed; [e]duration of clinical response = end time (time from drug intake when the difference between motor score and the baseline score ≤10% of the baseline score) – starting time; [f]sum of all the 13 AIMS scores over study visit; [g]highest AIMS score during the study visit. The figures in parentheses indicate the number of assessments in each group. Control vs. study treatments: *, $p < 0.05$; **, $p < 0.01$.

5.6.2. Dyskinesias during the L-dopa test (II)

In comparison to control therapy with L-dopa only, the mean increase in total daily AIMS score ("AUC" of dyskinesia) was 60% after entacapone, 47% after selegiline, and 95% after entacapone plus selegiline as L-dopa adjuncts in 14 PD patients (Table 19, Fig. 12), but only the difference between control and entacapone plus selegiline was significant ($p < 0.01$). Differences in total daily AIMS score between any two study treatments were non-significant. Differences in peak AIMS score were not significant between study treatments, or between each study treatment and control (Table 19).

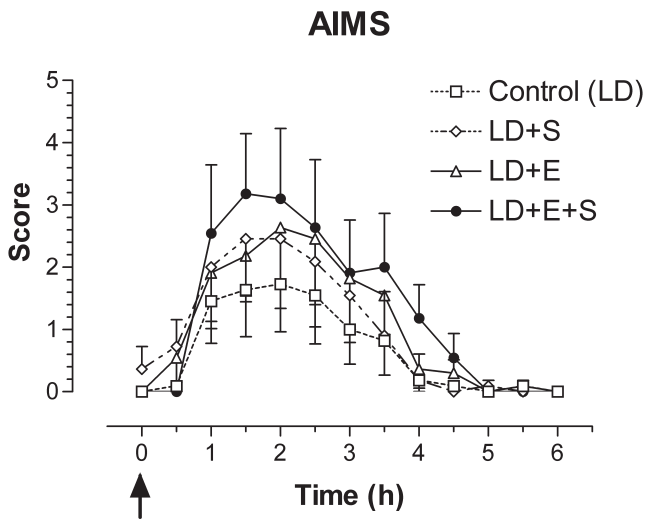


Fig. 12. Scores (mean ± SEM) for Abnormal Involuntary Movement Scale (AIMS), assessed first before (0 h), and then repeatedly after intake of study drugs (arrow) (II). The first assessment was done after intake of L-dopa (Control), and then after 2 weeks of L-dopa with selegiline 10 mg o.d. (LD+S), entacapone 200 mg t.i.d./q.i.d. (LD+E) or entacapone plus selegiline (LD+E+S). Higher score indicates more dyskinesia. N=11 for each group. See Section 5.6.2. and Table 19 for analyses of results.

5.6.3. Effect of exercise on motor response (IV)

Both pre- and post-exercise UPDRS motor scores (mean ± SD) of 15 PD patients who performed a maximal work-conducted bicycle exercise test were lower after administration of L-dopa with either entacapone (13.9 ± 8.6 and 15.9 ± 11.5, respectively) or placebo (15.8 ± 6.2 and 16.8 ± 6.2, respectively) than were the scores assessed during a run-in visit after overnight drug withdrawal (22.7 ± 12.5 and 24.8 ± 12.5, respectively). Within each group a trend emerged toward a slight but non-significant deterioration in motor function after exercise.

5.6.4. Sleep (I, II)

Differences in either the duration or quality of sleep (Table 20), based on the analysis of sleep actigraphy recordings, were not significant between any two study treatments or between study treatments and control (I, II).

Table 20. The duration and quality (mean ± SD) of sleep based on ambulatory activity monitoring (sleep actigraphy) (I, II).

Sleep assessment with actigraphy	*Treatment*			
	LD (control)	LD + E	LD + S	LD + E + S
Duration of sleep (h)				
• Study 1 (N=12-13)	6.3 ± 1.2	6.8 ± 1.7	NA	6.3 ± 1.5
• Study 2 (N=12-14)	6.3 ± 1.2	6.5 ± 1.1	6.2 ± 1.3	6.7 ± 1.4
Activity during sleep[a] (counts/epoch)				
• Study 1 (N=12-13)	41.8 ± 60.0	43.2 ± 46.4	NA	39.9 ± 22.2
• Study 2 (N=12-14)	43.6 ± 39.4	48.1 ± 32.8	44.3 ± 30.0	47.4 ± 32.6

Assessments were done first after L-dopa (control), and then after 2 weeks on L-dopa plus entacapone 200 mg t.i.d./q.i.d., L-dopa plus selegiline 10 mg o.d. (study 2 only), and L-dopa plus entacapone plus selegiline. LD, L-dopa; E, entacapone; S, selegiline; NA, not applicable. [a]mean motor activity from "lights-out" to awakening quantified in epochs of 25 sec. duration, based on wrist-worn accelerometric assessment (actigraphy).
No significant differences between treatments.

5.7. Adverse events (AE)

Of the most common AEs encountered in the first and second studies, most were transient and either mild to moderate in severity (Table 21).

In the first study, of 12 patients treated with entacapone plus selegiline as L-dopa adjuncts, 9 (75%) reported ≥1 AE, as compared to 9 (69%) of 13 of those receiving entacapone plus placebo. The total number of AEs during these treatments was also comparable, with 25 reported events during selegiline and 26 during placebo periods. Three patients (25%) experienced nausea during treatment with selegiline, whereas no nausea occurred during placebo (Table 21).

Table 21. The most frequently reported adverse events in Parkinsonian patients (I, II).

Adverse event	*First study*		*Second study*		
	LD+E (n=13)	LD+E+S (n=12)	LD+E (n=14)	LD+S (n=15)	LD+E+S (n=16)
Fatigue	4 (31%)	2 (17%)	7 (50%)	6 (40%)	2 (13%)
Dizziness	4 (31%)	3 (25%)	1 (7%)	1 (7%)	4 (25%)
Nausea	0	3 (25%)	1 (7%)	0	3 (19%)
Headache	4 (31%)	2 (17%)	0	1 (7%)	2 (13%)
Orthostatic hypotension	0	0	2 (14%)	5 (33%)	2 (13%)
Abdominal pain	2 (15%)	2 (17%)	0	0	1 (6%)
Diarrhea	0	2 (17%)	1 (7%)	1 (7%)	0
Insomnia	0	1 (9%)	0	2 (13%)	4 (25%)
Loss of appetite	1 (8%)	2 (17%)	0	0	1 (6%)
Dry mouth	1 (8%)	0	0	2 (13%)	2 (13%)
Patients with adverse events	9 (69%)	9 (75%)	8 (57%)	11 (73%)	9 (56%)

Occurrence (n) and frequency (% of patients) of the most common (occurring in ≥2 patients during any treatment period) adverse events during the 2-week treatment periods, as well as number of patients reporting adverse events. LD, L-dopa; E, entacapone; S, selegiline. Between-group differences in number of patients reporting adverse events non-significant.

In the second study, of 14 L-dopa treated patients, 8 (57%) reported ≥1 AE during the 2 weeks on entacapone, 11 (73%) of 15 during treatment with selegiline, and 9 (56%) of those 16 receiving treatment with both entacapone and selegiline. These differences in number were non-significant. The total number of AEs reported during study treatments was 21 for entacapone, 38 for selegiline, and 33 for entacapone plus selegiline. Loss of sleep was reported by two (13%) patients during selegiline therapy without entacapone and by four (25%) during selegiline with entacapone. No sleep loss occurred during the period with entacapone as the sole adjunct to L-dopa. Fatigue occurred least frequently during treatment with entacapone plus selegiline (Table 21), whereas dizziness and nausea were most frequently encountered in this group of patients.

In the third study, more ($p < 0.05$) patients reported AEs during the week on entacapone (n=12) than during the week on placebo (n=6). Two patients had nausea during entacapone, none during placebo. The number or type of AEs during exercise did not differ significantly between treatments. No severe AEs occurred.

6. DISCUSSION

This thesis is based on the results of two interaction studies on the combined use of entacapone and selegiline as adjuncts to L-dopa (I, II), and on the results of one trial on cardiovascular autonomic function (III) and cardiorespiratory exercise safety and capacity (IV) after repeated dosing of entacapone in L-dopa-treated patients with PD. All three studies had a controlled, double-blind, crossover design in order to control for the placebo effect and to avoid other sources of bias, either observer- or group-based. Washout periods used in two of the studies minimized carry-over effects. In general, the study methodology was well standardized. In each study, the patients selected were considered to represent "typical" cases of PD, and formed a rather homogeneous group in relation to disease severity and antiparkinsonian treatment. The original study protocols were followed rigorously with no major deviations.

A diverse array of methods and parameters (biochemical, pharmacological, metabolic, physiological, clinical) were applied. As a downside, the protocols were rather complicated and time-consuming, thus limiting sample size and statistical power. Duration of treatment was measured in days to weeks, thus limiting any conclusions as to the potential effects of chronic treatment. In addition, the pharmacodynamic effects of selegiline recede slowly, resulting potentially in a considerable carry-over effect.

6.1. Cardiorespiratory aspects

6.1.1. Hemodynamics and cardiac rhythm

Some studies show COMT inhibition to alter hemodynamics despite inducing no change in the plasma levels of catecholamines. In healthy humans, entacapone potentiates the chronotropic effect of isoprenaline without affecting its plasma concentration (277). In patients with MSA, entacapone has induced a marked hypersensitivity to phenylephrine compared to its effects in healthy controls, in addition to eliciting a moderate and dose-dependent increase in systolic BP in MSA patients, despite unchanged plasma levels of DA, NA, and adrenaline (280). It can therefore be hypothesized that COMT inhibitors may have differential effects on plasma and receptor-level concentrations of catecholamines, and that their hemodynamic effects may be altered in subjects with significant cardiovascular autonomic dysfunction. According to a microdialysis study in experimental animals, entacapone did not change the myocardial interstitial levels of NA, although significant increases and decreases occurred in its respective MAO- (DHPG) and COMT-dependent (normetanephrine and MHPG) metabolites (307).

Similar to the use of either entacapone or selegiline as a sole adjunct to L-dopa, the simultaneous use of these two drugs as L-dopa adjuncts did not significantly change hemodynamics such as daily supine/standing BP or HR (I, II). The chronic use of selegiline has been reported to diminish sympathetic autonomic responses (166) and to be associated with orthostatic hypotension in L-dopa-treated PD patients (308). According to the results from the current studies, 2 weeks on selegiline – either with or without entacapone – did not enhance the orthostatic fall in BP of L-dopa treated patients. The combination therapy with these two drugs was hemodynamically well tolerated, which is in accordance with the results from other double-blind, randomized, placebo-controlled phase II studies

(234, 239), and also from phase III studies on entacapone (10, 11, 248, 259), in which a significant number of patients were also taking selegiline. The 6-month Nordic study with nearly half the patients using selegiline detected no significant differences in supine or standing systolic BP or HR between the entacapone and placebo treatment arms (11). In the 12-month safety study with over 300 PD patients, mean orthostatic fall in systolic BP and overall tendency to orthostatic hypotension did not differ between entacapone and placebo, despite the frequent (> 80%) use of selegiline in both groups (259). Similar results were obtained in the German-Austrian multicenter study in which use of selegiline was less (52–56%) common (248).

Although the cardiac safety of entacapone has been extensively studied in parkinsonian patients, for instance with intermittent ECG recordings, a more sensitive method for detecting cardiac rhythm disturbances, ambulatory ECG (Holter), has not been used for this purpose. Ambulatory ECG provides, however, a better possibility to assess cardiac rhythm in differing circumstances, e.g., at rest, during effort, and – in the case of PD patients – during "on" and "off" states. The present study revealed that neither entacapone nor selegiline, administered either separately or together, had any effect on mean HR or the occurrence of cardiac arrhythmia during a 24-hour ECG recording (II, IV). This is in agreement with the clinical safety data from larger phase III studies on entacapone, in which no ECG changes or clinically significant cardiac AEs occurred during 6-month treatment, regardless of the frequent use of selegiline as a co-adjunct therapy (10, 11). According to the prospective 6- to 12-month safety studies in which selegiline was also used by about half the patients, the proportion of newly emerging ECG abnormalities between the entacapone and placebo arms was comparable (248, 259). In a 3-year open-label extension of the phase III Nordic study, no ECG abnormalities occurred with entacapone (251). Findings similar to the present results have also been reported with tolcapone as add-on therapy to L-dopa. Based on data from 24-h ECG recordings, no change in the number of arrhythmic events was observable after 6 months on the drug (281). In an open-label study, 2 weeks of tolcapone (mean daily dose of 271 mg) had no effect on the 24-hour ambulatory HR of eight patients in comparison to matched controls not taking the drug (274).

Although several safety studies on entacapone have investigated its effects on exercise hemodynamics in healthy volunteers (16, 17, 271, 272), the present study (IV) is the first to investigate the exercise safety of the drug in L-dopa-treated parkinsonian patients. Neither ECG nor hemodynamic (BP and HR) responses to maximal exercise changed after repeated dosing of entacapone for one week as an add-on to L-dopa. Current findings are in accordance with those from studies in healthy volunteers, and are also consistent with long-term experience in the cardiovascular and hemodynamic safety of entacapone in parkinsonian patients (170, 259). It is also noticeable that all patients participating in the present study were on selegiline therapy, which suggests that no exercise-induced hemodynamic or cardiac rhythm problems are associated with the concomitant use of these two L-dopa adjuncts in PD patients.

6.1.2. Cardiovascular autonomics

In an open-label study by Myllylä et al., a single dose of entacapone had no effect on cardiovascular autonomic responses to sympathetic and parasympathetic stimuli (235). Prior to the present study (III), however, no double-blind, placebo-controlled trials have been performed on the effects of repeated administration of entacapone on cardiovascular autonomic function in PD patients. Such an investigation seemed prudent, because many PD patients demonstrate dysfunction of cardiovascular autonomics and because of

theoretical safety issues related to inhibition of COMT, due to its role in the function of the sympathetic nervous system. In the present study, no clinical evidence of cardiovascular autonomic dysfunction was sought prior to recruitment. In a post-hoc comparison of HR responses with reference values from a healthy Finnish population (287), no clinically significant cardiovascular autonomic dysfunction seemed to occur in any patients.

Hemodynamic responses to physicochemical alterations in external/internal milieu are due to joint actions of the sympathetic and parasympathetic divisions of the cardiovascular autonomic nervous system. Conventional cardiovascular autonomic reflex testing is a simple and clinically useful method of assessing these responses (285). A battery of tests provides the possibility to discriminate between sympathetic and parasympathetic dysfunction, and is also useful in the differential diagnostics of parkinsonian syndromes (309).

Repeated dosing of entacapone as an adjunct to L-dopa had no effect on any of the parameters of cardiovascular autonomic function, such as HR variation during deep breathing, the Valsalva maneuver, or orthostatic challenge, results in line with those of the previous open-label, single-dose study with comparable methodology (235). Entacapone had no significant effect either on BP response during orthostatic testing or on frequency of orthostatic hypotension, in accordance with the results from the 6- to 12-month, double-blind, placebo-controlled phase III studies (10, 11, 248, 259) and the 3-year open-label study (251), in which neither clinically relevant findings for BP, HR, nor any accentuation of orthostatic hypotension was associated with use of entacapone. It could be argued that the lack of effect of COMT inhibition on the autonomic parameters in the present study is in part due to the predominantly parasympathetic control of these responses. However, no potentiation of diastolic BP increase in response to isometric effort occurred after entacapone, although this parameter mainly correlates with level of sympathetic (noradrenergic) activity. These results therefore suggest that peripheral COMT inhibition is devoid of any clinically significant effects on cardiovascular autonomic function. The question whether changes in these variables can be detected by using a larger number of patients or more accurate and reproducible methods, such as 24-h ambulatory ECG with analysis of spectral components of HR variation (52, 310) remains unanswered.

Data are scarce on the effects of tolcapone upon cardiovascular autonomic responses. In one study, HR variability based on 24-hour ECG did not change after 6 months on the drug (281).

Several anti-parkinsonian drugs have been reported to modulate cardiovascular autonomic function: The effects of L-dopa (Section 2.2.3.), in particular, remain controversial (18, 43, 61, 166, 308). Although the present results showed an increased frequency of orthostatic hypotension after L-dopa (both with and without entacapone) when compared to the overnight withdrawal of the drug, no significant differences occurred in mean changes in BP or HR during the orthostatic test. Although both selegiline (61, 166, 308) and DA agonists (61, 163) have been reported to have effects on cardiovascular autonomic function, for instance, aggravation of orthostatic fall in systolic BP (Section 2.2.3.), responses here seemed not to be biased by these drugs, based on the cross-over design of the trial.

6.1.3. Cardiorespiratory exercise performance

Enhanced muscle fatigue, abnormally low peak power (311, 312), reduced metabolic efficiency of work on the more affected side (313), ventilatory dysfunction (314–316), and cardiovascular autonomic dysfunction (Section 2.1.2.) may all contribute to the impaired

cardiovascular competence suggested to occur in PD. Physical fatigue in PD has been at least partially related to deficiency in DA (317). L-dopa has been reported to improve ventilatory function (318), exercise endurance, metabolic efficiency of work (319), and physical fatigue in finger tapping and force generation (317).

The present study enrolled only those patients with a relatively mild motor disability, because patients with advanced disease and significant motor disability are presumed to have considerable, if not overwhelming, difficulties in coping with maximal physical effort, particularly during the first test after overnight drug withdrawal. No specific requirements for physical fitness were set, however, and both sedentary and physically active patients were enrolled.

In each case, the maximal level of exercise was evaluated with the use of subjective (Borg's scale), metabolic (RER), and circulatory (age-predicted maximal HR, = 205 – age/2) criteria. According to subjective and metabolic criteria, an adequate (maximal) level of physical effort was achieved in every case. However, the circulatory criterion of exercise maximum was reached by only six (40%) of the patients during every test, whereas seven (47%) patients never reached it. That both "blunted" (320, 321) and normal (311, 322) HR responses to exercise in comparison to age-predicted maximal HR have been reported in parkinsonian patients indicates that the age-predicted maximal HR may not be as valid a measure of maximum exercise level in PD patients as it is in healthy humans, possibly due to such factors as cardiovascular autonomic incompetence. Because age-predicted maximal HR has inherent drawbacks, for instance, measurement error and inter-subject variability, it is, according to some, an inaccurate indicator maximum exercise level (323).

Antiparkinsonian drugs (L-dopa plus selegiline, either with or without entacapone) had no influence on HR response to exercise when compared to overnight withdrawal of treatment. Similar findings of an unchanged HR response between pre- and post L-dopa bicycle exercise testing exist (319).

Entacapone did not change the effect of L-dopa on maximum workload; higher values for maximum workload were achieved after L-dopa – regardless of entacapone – than after overnight withdrawal of antiparkinsonian drugs. This finding could be interpreted to be in agreement with findings on L-dopa's improving exercise endurance (319). Other factors: placebo and the "training" effect (familiarization with the test procedure) may also contribute to such an improvement in work performance.

Entacapone also had no effect on maximum O_2 uptake of L-dopa-treated patients. Although a significant increase occurred in maximum O_2 uptake after L-dopa plus placebo in comparison to control values, no definite conclusions on contrasts between control and study treatments can be drawn, due to the design of the study (unblinded control, fixed temporal relation of run-in and other test days). The increased maximum O_2 uptake may have been due to training or other effects, because the maximal workload was also higher after study treatments than during control.

In healthy humans, ventilation is not a limiting factor for maximal exercise performance; instead there exists a ventilatory reserve at peak exercise (defined as the difference between maximal voluntary and exercise ventilation), also called the breathing reserve. In the healthy, this comprises approximately 20 to 40% of maximal voluntary ventilation. Low values for breathing reserve are observable in restrictive lung disorders, for instance, but also in individuals with extremely high cardiovascular competence. The breathing reserve may be abnormally high also when exercise performance is limited by cardiovascular disease. In the calculation of breathing reserve, maximal voluntary ventilation must be determined, either by direct measurement or estimation. In neurological disorders, indirect estimates may lead to an overestimation of actual maximal voluntary ventilation

(324). Such may also be the case in PD, which can demonstrate impaired performance in repetitive ventilatory efforts (314, 315), so direct measurement of 15-second maximal voluntary ventilation was therefore chosen. Entacapone had no effect on maximum exercise ventilation or on the breathing reserve of L-dopa-treated patients, and neither of these parameters was altered by L-dopa (with or without entacapone) in comparison to control. These results therefore suggest that neither drug changes the ventilatory response to maximal exercise.

The effect of work rate was eliminated by comparing cardiorespiratory parameters at standard (submaximal) workload, at which O_2 uptake is a measure of exercise efficiency. This remained unchanged, irrespective of whether the patients were "off-the-drug" (run-in) or in the ON-phase after L-dopa. This finding somewhat contradicts one report on L-dopa's improving metabolic work efficiency (319). Overnight drug withdrawal does not, however, necessarily represent a true off-state, as L-dopa also has a long-duration effect (325). Nor can the dopaminergic effects of longer-acting drugs like DA agonists and selegiline be excluded. Entacapone may, at least theoretically, enhance the putative beneficial effects of L-dopa on exercise performance and efficiency, although the standard O_2 uptake of L-dopa-treated patients remained unchanged by entacapone.

The standard O_2 pulse was slightly but significantly lower after entacapone than after placebo. Although the significance of this finding remains unknown, among the alternatives, one – probably the most likely – is the occurrence of a statistically "false-positive" finding. A less likely one is a true drug effect. The O_2 pulse is a compound variable, equaling the product of stroke volume and arteriovenous O_2 difference. It is unlikely that peripheral COMT inhibition would cause any reduction in stroke volume. A decrease in the peripheral component of O_2 pulse (arteriovenous O_2 difference), due perhaps to redistribution of blood flow or decreased tissue O_2 extraction or both, is another possibility. Whether or not entacapone can induce such changes remains unresolved. The difference in O_2 pulse between treatments was minor and not clinically significant.

Ventilatory equivalents for O_2 or CO_2 are indices of ventilatory efficiency. Abnormally high (=inefficient) values for these quotients are observable during hyperventilation and in states of pathological mismatch between alveolar ventilation and capillary perfusion. Here, that entacapone had no effect on ventilatory equivalents for O_2 or CO_2 suggests that peripheral COMT inhibition does not alter ventilatory response to submaximal workload.

In summary, the present findings suggest that in L-dopa-treated PD patients, entacapone – used either in combination with or without selegiline – has no clinically significant effect on resting/exercise levels of plasma catecholamines, on hemodynamics, on cardiovascular autonomic function, or on cardiorespiratory exercise performance.

6.1.4. Plasma catecholamines

Repeated dosing of entacapone 200 mg with each dose of L-dopa for 2 weeks did not change the plasma levels of catecholamines (either NA or DA) in 13 PD patients (I), but the metabolic profile of NA was altered, as demonstrated in the significantly decreased plasma level of its COMT-dependent metabolite MHPG. These findings are in agreement with previous theories as to the effects of COMT inhibition on methylated catechol metabolites (156), and also with findings in healthy human volunteers (16, 17, 271, 272, 326). MHPG is formed extraneuronally, either by the sequential actions of MAO and COMT on NA or by O-methylation of intraneuronally produced DHPG (327). The effects of acute alterations in neuronal NA release on plasma MHPG are relatively minor, whereas the decreased plasma levels of this metabolite have been suggested to accurately reflect

pharmacological inhibition of COMT (326, 328). Findings of unchanged plasma levels of catecholamines together with their altered metabolic profile during COMT inhibition are consistent with the presumption that parallel catabolic pathways do compensate for the reduced COMT activity. Based on this concept, COMT inhibition results in shunting of catecholamine metabolism towards the MAO pathway.

The effects of tolcapone on plasma catecholamine levels may differ from those observed with entacapone. A study of L-dopa-treated PD patients by Rojo et al. demonstrated a significant (more than six-fold) increase in mean plasma levels of DA, NA, and adrenaline after 2-week use of the drug (274). The authors speculated that such differences in the effects of entacapone and tolcapone on plasma catecholamines may be due to the greater potency of tolcapone. In our opinion, however, the data of this particular study may have been interpreted erroneously, and the results have not been confirmed in other trials.

In the present study, repeated doses of selegiline 10 mg had no effect on plasma levels of DA or NA, nor did it alter the effects of entacapone on plasma MHPG in L-dopa-treated parkinsonian patients (I). These results are consistent with this drug's MAO-B selectivity – and therefore its minor contribution to the peripheral catecholamine metabolism – at this dose level (152, 329). In peripheral tissues and plasma, no appreciable amounts of MHPG seem to be derived from MAO-B-dependent deamination (326, 327). The situation has differed with co-inhibition of MAO-A by moclobemide and COMT by entacapone (271). In that case, MAO-A seems to be the main contributor to alterations observed in the metabolic profile of NA (mainly decreased plasma MHPG), although plasma levels of free catecholamines had remained unchanged.

During strenuous exercise, a marked (at least ten-fold) increase was apparent in plasma levels of free catecholamines such as NA. However, substantial inhibition of peripheral COMT by entacapone had no effect on peak exercise concentrations of the plasma NA of L-dopa-treated parkinsonian patients (IV). Findings on the effects of both entacapone and nitecapone on levels of plasma catechols during exercise performed by healthy human volunteers have been in accordance with those results (16, 17, 269-271). The effects of tolcapone on plasma catecholamines during exercise have not yet been documented.

6.2. COMT activity and L-dopa pharmacokinetics

After repeated administration of 200 mg of entacapone with each daily dose of L-dopa for 2 weeks (I), a highly significant reduction in erythrocyte S-COMT activity occurred, measured one hour after the dose, when the inhibitory effect of the drug should be at its highest (231). S-COMT activity was reduced by 36 to 38% from baseline (before entacapone). Although somewhat higher levels of inhibition at this dose level have appeared in healthy volunteers (231, 242), the present results are in line with other findings in PD patients, in which a similar level (33-38%) of inhibition of S-COMT activity occurred after entacapone 200 mg (232, 233). COMT activity in erythrocytes was unaffected by selegiline, as previously reported (330).

In line with the effective COMT inhibitory activity of entacapone after repeated doses, bioavailability (AUC) of L-dopa was much improved (I) without significant changes in its absorption kinetics (C_{max} and T_{max}). These results are in agreement with the majority of pharmacokinetic results in healthy human volunteers (236) and PD patients (204, 232-235). Although some of these were single-dose studies, others have demonstrated that the changes in L-dopa pharmacokinetics induced by entacapone remain unaltered from day to day during its repeated administration (239, 241).

The published effects of entacapone on the $t_{1/2}$ of L-dopa have varied, possibly due to differences in properties of study subjects, frequency/duration of sampling, or variable absorption after oral doses. No change in the $t_{1/2}$ of L-dopa at the 200-mg dose level of entacapone has been observed by some (236, 239, 242), but findings in parkinsonian patients have been more consistent with a significant increase in the $t_{1/2}$ of L-dopa after entacapone (233, 235, 240). This increase has been most prominent after intravenous administration of L-dopa (204), because in such circumstances the "true" elimination $t_{1/2}$ of the drug (vs. "apparent" $t_{1/2}$ with oral dosing) can be measured. The present study (I) showed no clear increase in the $t_{1/2}$ of L-dopa caused by entacapone, possibly because the study was not primarily designed for pharmacokinetic purposes. Selegiline had no effect on the pharmacokinetic parameters of L-dopa, consistent with previous experience (331).

In accordance with experience from other repeated-dosing studies (204, 233, 234, 239), prolonged administration of entacapone caused a highly significant decrease in the formation of 3-OMD, the peripheral COMT-dependent metabolite of L-dopa. This sustained decrease has also been evident in long-term therapeutic trials (11). The daily plasma levels of 3-OMD remained quite stable during repeated assessment, also consistent with earlier results (204, 243) and with the long peripheral half-life of this metabolite (199). Due to its stable plasma levels, 3-OMD – unlike highly variable plasma L-dopa and COMT activity – is a good measure both of treatment compliance and of the pharmacological effect of COMT inhibitor therapy.

Consistent with available data (14), the peripheral metabolic profile of DA was significantly affected by concomitant administration of entacapone with L-dopa: plasma levels of DOPAC (the MAO-dependent metabolite of DA) were significantly increased, reflecting a shift in the peripheral metabolism of DA away from COMT and towards MAO-dependent oxidation. The majority of clinical studies have reported the same for either healthy humans (236, 238, 242) or parkinsonian patients (232, 235, 240, 243, 244). The increased levels of DOPAC during peripheral COMT inhibition may be the result of an increased peripheral decarboxylation of L-dopa to DA and further to DOPAC (14).

Effects of entacapone on plasma HVA (end-metabolite of DA) levels have varied more. Some (primarily single-dose) studies have not shown entacapone to alter plasma HVA (236, 247). Its plasma levels were, however, significantly reduced by entacapone in the current study, consistent with the the majority of clinical trials (232, 234, 238, 240, 243).

Although experimental animal data on interactions of entacapone and selegiline in brain DA metabolism are available (218), clinical studies on the combined effects of these drugs on peripheral DA metabolism are lacking. It is clear that DA metabolites derived from various sources (brain and peripheral tissues) are mixed in plasma, and their origin cannot be traced (192). According to some experiments, however, most plasma HVA is extracerebral, derived from MAO-A-dependent deamination of DA in the peripheral neurons, and that plasma DOPAC is also primarily derived from cells outside the central nervous system. The plasma levels of these metabolites can thus probably not serve as indices of central DA (including MAO-B mediated) metabolism (188, 331-333). MAO-B-selective doses of selegiline should, therefore, have no effects on the metabolic profile of peripheral DA. In line with these ideas, only a negligible effect of selegiline on plasma DOPAC has been demonstrable (331, 334). In the present group of PD patients, selegiline did not modify the effects of peripheral COMT inhibition on plasma HVA levels, consistent with animal data (332). However, selegiline significantly attenuated the entacapone-induced increase in plasma DOPAC. Assuming that the majority of plasma DOPAC derives from peripheral sources, this finding therefore seems to indicate that in the presence of

significant peripheral COMT inhibition, selegiline is able to modulate the metabolic profile of peripheral DA.

Entacapone demonstrated rapid absorption kinetics, with a T_{max} value of approximately one hour. The pharmacokinetic parameters of the active drug (E-isomer) were consistent with those previously seen in healthy volunteers (231, 335). Entacapone also has one pharmacologically active metabolite, the Z-isomer. In the present study, the AUC of this metabolite accounted for some 6 to 7% of the total plasma AUC of both isomers, in accordance with that of other reports (157, 302). That selegiline had no effect on the plasma levels of either entacapone or its Z-isomer demonstrates the lack of pharmacokinetic interactions between these two drugs.

In summary, entacapone caused a significant reduction in peripheral COMT activity and an improvement in L-dopa bioavailability, which is in agreement with available pharmacokinetic data. Its effects on the metabolites of L-dopa and DA were also consistent with those from previous studies. L-dopa $t_{1/2}$ in this study was not clearly increased. Although selegiline had no effect on the pharmacokinetic parameters of L-dopa, it seemed – in the presence of simultaneous COMT inhibition – to alter the metabolic profile of peripheral DA in a manner consistent with its MAO-B inhibitory action. Selegiline had no effect on the pharmacokinetics of entacapone.

6.3. Clinical response to L-dopa

Although the efficacy of entacapone (10, 11, 169, 170, 239) and selegiline (336, 337) as adjuncts to L-dopa in PD patients with motor response fluctuations is established, the clinical effects of their combined administration with L-dopa has not been studied systematically. Data from several long-term studies suggest that entacapone is efficacious in enhancing the clinical effects of L-dopa independent of the extensive usage of selegiline as co-adjunct therapy (10, 11, 248, 251).

A wide variety of methods and scales have been applied in the assessment of antiparkinsonian drug efficacy. A composite of various scales for assessment of parkinsonian disability, disease progression, efficacy, and complications of antiparkinsonian therapy, the UPDRS has good inter-rater reliability and validity for assessing motor response to L-dopa in PD patients (79, 338-340). The same applies to the 14-item motor subscale (part III) of UPDRS (341), although it has been subject to interference from dyskinesia and dystonia (342). The present studies used a slight modification of the motor subscale (234). The present definition of the onset of motor response (>10% decrease in motor UPDRS from baseline) was similar to the ones documented previously (343-345), and also with those in other clinical studies on entacapone (232, 239, 243).

In parallel with an increase in L-dopa bioavailability, a significant improvement in daily motor disability appeared in one of the studies (I) after the addition of entacapone to L-dopa. This is in accordance with results from other phase-II clinical studies with comparable methodology and parameters of clinical efficacy (234, 243). In the second study (II), the differences in mean daily motor scores between control and study treatments were non-significant, although adding each study treatment to L-dopa led to some improvement in the other measure of motor disability over the 6-hour time window. Due to the unblinded control assessment, however, these secondary statistical comparisons are subject to bias.

Selegiline seemed to induce a mild enhancement in the clinical response to entacapone plus L-dopa, suggesting an additive therapeutic effect of the combined COMT and MAO-B inhibition on L-dopa efficacy, possibly through the increased entry to and

prolonged action of L-dopa in the brain (I). In the second study (II), however, combined entacapone and selegiline demonstrated no clinical superiority over either adjunct alone, regardless of the efficacy parameter. These results are therefore also inconclusive. It can be speculated that a significantly larger number of patients is required to show any meaningful difference in the efficacy of these compounds for PD patients on the "optimal" (meaning providing the best possible symptom control) therapeutic regimen. An alternative would be to study patients on sub-optimal L-dopa dosage.

In the present study, duration of clinical response to L-dopa was affected by neither separate nor combined administration of entacapone and selegiline, in contrast with the other results in PD patients with end-of-dose type motor fluctuations, in which entacapone has induced a significant increase in ON-time (10, 11, 204, 240, 249). Several explanations for this discrepancy and the lack of effect here on duration of motor response are conceivable. One is the small sample size, i.e., lack of power. The other is the characteristics of the patients; although all had a history of end-of-dose-type motor fluctuations, the presence of these fluctuations de facto was not objectively verified prior to inclusion. A post hoc analysis of UPDRS motor subscores from the second study (II) showed that it was conceivable that approximately half the patients demonstrated a rather stable short duration response to L-dopa. If motor fluctuations were actually present in these patients, they were rather subtle and not clinically evident. Such a large number of clinically non-fluctuating patients could well negate the identification of a potentially positive drug response. A need for a more accurate pre-trial evaluation of the state of the disease is therefore apparent in clinically mildly affected patients. Finally, there was a tendency for a reduced (better) "baseline" motor disability in the mornings after each study treatment in comparison to control. This finding could be due to placebo effect or true overnight extension of dopaminergic effects of the investigated drugs ("sleep benefit"). This difference in baseline motor disability seemed to result in an apparent exaggeration of drug response in favor of control. The lack of effect of entacapone on time of onset or magnitude of clinical response to L-dopa in the present study is in accordance with both the pharmacokinetic data and other clinical experience with the drug (158).

Both entacapone (10, 11, 234, 239, 259) and selegiline (146, 152) may promote dyskinesia. In the present study, an aggravation of dyskinesia was therefore anticipated after addition of either one or both of these L-dopa adjuncts to patients on an "optimal" antiparkinsonian regimen. As expected, dyskinesia seemed to be most prominent after the combination of entacapone and selegiline. In comparison to plain L-dopa (II), a significant increase in the mean for daily dyskinesia (AIMS) occurred only after co-administration of the two adjuncts. A review by Kaakkola includes reports of this propensity for an increase in dyskinesia during entacapone plus selegiline co-therapy with L-dopa (157). Similar dyskinesia scores between active study treatments suggest that the co-administration of entacapone and selegiline does not result in a clinically significant augmentation of dyskinesia compared with the use of either drug alone with L-dopa. L-dopa dosage reductions due to dopaminergic AEs were, however, made before assessment of dyskinesia, possibly biasing the results by, for instance, diminishing differences between treatments. After co-admistration of entacapone and selegiline with L-dopa, the peak dyskinesia score was not higher.

In summary, selegiline seemed to slightly enhance the clinical response to entacapone plus L-dopa combination therapy in the first study, but not in the second. Several factors such as limitations in study power and patient characteristics could partially explain some discrepancies. Entacapone had a propensity to lead to increased dyskinesia, most prominently after co-administration with selegiline.

6.4. Tolerability

The present study indicates that the co-administration of selegiline and entacapone as L-dopa adjuncts is safe and well tolerated, in agreement with the clinical safety data from several phase II (204, 234, 235, 239, 240) and phase III studies on entacapone (10, 11, 248, 251, 259), in which a considerable number of patients were on selegiline co-adjunct therapy. The co-administration of tolcapone and selegiline as add-on therapy to L-dopa has also been reported to be safe and well tolerated (282), although one study showed a decrease in tolerability due to diarrhea, nausea, and dizziness after a combination of these two drugs for otherwise untreated patients (262).

A tendency appeared toward an increased number of dopa-related AEs after combination of entacapone and selegiline with L-dopa. This was anticipated because the dopa-potentiating effects of these two adjuncts are well known (10, 146, 152, 234, 248), and because the antiparkinsonian therapy was already adjusted to provide the best possible symptomatic effect. Nausea, in particular, seemed to occur more frequently during co-administration of entacapone and selegiline. Insomnia and sleep-related problems seemed to be associated with selegiline, irrespective of entacapone use, in accordance with clinical experience with this drug (149). Although selegiline has been suggested to influence sleep through non-dopaminergic mechanisms (346), the present findings of a further increase in frequency of insomnia after a combination of the two L-dopa adjuncts suggests that dopaminergic mechanisms are also involved. In a 12-month double-blind safety study with 326 patients, however, insomnia occurred at approximately equal frequency during entacapone and placebo, despite the frequent (>80%) use of selegiline in both treatment arms (259).

In two of the studies (I, II), L-dopa dosage reduction was necessary in three of 29 patients after combination of entacapone and selegiline with each other. In each case, this was due to emergence of dopaminergic AEs: dystonia or dyskinesia. In the third study (III, IV), in which all the patients were on concomitant selegiline therapy, L-dopa dosage was reduced after introduction of entacapone in two of 15 patients. In each case, dopa-related AEs were effectively controlled by these dosage reductions. A re-institution of pre-trial L-dopa dosage was required in four of the patients within days after entacapone withdrawal because of aggravation of parkinsonian symptoms. This worsening of disability upon such withdrawal has been well documented clinically (10, 11, 248, 253, 259).

The two AE-related withdrawals in the studies were considered to be due to excessive dopaminergic potentiation by the two L-dopa adjuncts. In these patients, insomnia and dizziness were particularly likely to be treatment-related: These are the most common treatment-related AEs of entacapone (251), and have also been related to the use of selegiline (146, 152).

Dopaminergic therapy is one likely contributor to the sleep disorders in PD; selegiline has caused insomnia (149, 347). The effects of L-dopa on sleep are more controversial (64, 348-350). Ambulatory activity monitoring (actigraphy) techniques for assessment of drug effects on sleep (64, 350), when used in the present study, suggest that combined use of entacapone with selegiline in L-dopa-treated PD patients leads to neither deterioration in nor improvement of sleep, making more data necessary for confirmation.

In summary, the present results suggest an increased tendency for dopa-related AEs during the combined use of entacapone and selegiline as L-dopa adjuncts, but this combination of drugs was generally well tolerated, and dopaminergic AEs were, in most cases, controlled by reductions in daily dosage of L-dopa.

7. CONCLUSIONS

The main results of the present study are in line with other clinical experience on the use of entacapone as an L-dopa adjunct in parkinsonian patients. They indicate that:

- Co-administration of entacapone plus selegiline with L-dopa is safe and well tolerated in PD patients, independent of level of physical activity. No clinically relevant drug-drug interactions, such as cardiovascular AEs or potentiation of plasma catecholamine responses occurred.

- Entacapone had no effect on cardiovascular autonomic function.

- Entacapone had no effect on the profile of cardiorespiratory exercise performance.

- The clinical efficacy of L-dopa may be further improved by combining entacapone and selegiline as L-dopa adjuncts, although clinically significant potentiation of dopaminergic AEs, particularly dyskinesia, may also occur. Dopaminergic AEs were, in most part, effectively controlled by reducing L-dopa dosage.

8. ACKNOWLEDGMENTS

The present study was carried out at the Department of Neurology and at the Department of Clinical Physiology and Nuclear Medicine, Helsinki University Central Hospital. I wish to express my sincere gratitude to Professor Markku Kaste, Head of the Department of Neurology, Helsinki University Central Hospital, for his positive spirit, for encouragement, and for placing the excellent facilities of his Department at my disposal.

I express my deepest appreciation to my supervisor, Docent Seppo Kaakkola, for guidance, encouragement, and, foremost, overwhelming patience and good will. His thorough knowledge on clinical neuropharmacology, Parkinson's disease, and other movement disorders has been most inspiring. I should also like to express my sincere thanks to Professor Anssi Sovijärvi, my second supervisor, who has been easily approachable, in good spirits, and who provided guidance into the difficult field of clinical physiology, as well as use of the state-of-the-art facilities of the Department of Clinical Physiology and Nuclear Medicine.

I am also deeply grateful to Professor Heikki Teräväinen for starting all this and for demonstrating inspiring – and possibly unsurpassed – expertise in the field of clinical neurology, and Professor Ariel Gordin for his tireless support and encouragement essential for the accomplishment of this bi-millenial scientific work. Without these two gentlemen, this work would not exist.

I also wish to express my gratitude to Professor Pekka Männistö, whose pioneering work with the concept of selective COMT inhibitors has made this work possible.

Docents Tapani Keränen and Olli Raitakari are sincerely acknowledged for carefully reviewing the manuscript and for their constructive criticism.

I would like to express my sincere thanks to all my friends from the Department of Neurology, especially Docents Olli Häppölä, Markus Färkkilä, Seppo Soinila, Eero Pekkonen, Pentti Tienari, and Risto O. Roine, and colleagues Mikko Kallela, MD, PhD, Marjaana Tiainen, MD, and Elena Haapaniemi, MD, and many others who have kindly provided me with an enjoyable working environment, as well as enormous amounts of help and advice in the field of both clinical neurology and research, mixed adequately with a pleasant and humorous atmosphere.

I am also grateful to the former and present research team at Orion Pharma, especially Eeva-Riitta Kultalahti, Helena Heikkinen, Sirpa Ahtila, Kirsi Korpela, Pirjo Kovanen, and Mika Leinonen for kind assistance and pleasant teamwork throughout the years. Their work and effort was – not to exaggerate – indispensable.

I also wish to thank Carol Norris for skillful and rigorous author-editing of the language and for good advice, flexibility, and understanding.

I am also indebted to many of our collaborators, such as Professor Mika Scheinin for aid in various biochemical analyses, Päivi Tuomainen for COMT activity assays, and Docent Juhani Partanen for analyzing the ambulatory ECG recordings. I would also like to thank Samuli Ripatti from the Consultant Group Covariance Ltd., Helsinki, and the staff of Clinical Research Services, Turku, for statistical planning and analyses of the results.

I would like to thank the staffs of the Outpatient Department of Neurology and the Department of Clinical Physiology and Nuclear Medicine for their considerable and time-consuming effort in making this work possible.

My deepest gratitude goes to the patients for outstanding commitment and participation. Although huge scientific leaps are required in uncovering the mystery of – and hope-

fully a cure for – Parkinson's disease, the ever-steeper incline in the number of discoveries each year gives us high hopes for the future.

I owe enormous gratitude to my parents for their love and ongoing support.

Finally, my heartfelt gratitude to Mari for love, patience, encouragement, and support, and to my children Pekka and Anna for being there, and for keeping my priorities in balance.

This study was supported financially by the Research Funds of the Helsinki University Central Hospital, the University of Helsinki, the Research Institute of Orion Corporation, the Finnish Parkinson Foundation, the Finnish Medical Society Duodecim, the Finnish Medical Society, and the Finnish Neurological Society.

Helsinki, May 2006

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